UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE MEDICINA

DEPARTAMENTO DE PEDIATRÍA

Programa de doctorado en Medicina y Cirugía RD 99/201

"Treatment related deaths in childhood acute lymphoblastic leukemia"

Tesis Doctoral presentada por:

Francisco José Bautista Sirvent

Dirigida por:

Profesor Luis Madero López

Dr. Lucas Moreno Martín-Retortillo

El Profesor Dr Luis Madero López y el Dr Lucas Moreno Martín-Retortillo,

Certifican que la tesis doctoral que lleva por título "*Treatment related deaths in childhood acute lymphoblastic leukemia*" de la que es autor Don Francisco José Bautista Sirvent, licenciado en Medicina, se ha realizado entre las dependencias centrales de la European Organization for Research and Treatment of Cancer (EORTC) en Bruselas, Bélgica, y el Hospital Niño Jesús de Madrid, España, bajo nuestra supervisión y estimamos que reúne los requisitos necesarios para optar al título de doctor, destacando la contribución positiva del planteamiento tal y como se refleja en las conclusiones alcanzadas.

Y para que conste a los efectos oportunos, firmamos el certificado en Madrid a 21 de Abril de 2016.

Profesor Luis Madero López

Doctor Lucas Moreno Martín-Retortillo

A Estefanía y a Matilde

ACKNOWLEDGEMENTS

A mis padres y mi hermano, quienes siempre han apoyado mis iniciativas personales y profesionales, y han sabido darme el ánimo y el estímulo necesario para llevarlas a buen puerto.

A Estefanía (y ahora también a Matilde, la sonrisa de cada día). Por ser tan paciente y aguantar todos los momentos que dedico al trabajo. Por ser tan buena, y soportar mis ratos malos. Por ser tan alegre, y hacer felices cada uno de los días que paso a su lado. Sin ella(s), esto no estaría siendo posible.

A Amparo y Chema, mis adjuntos de oncología en la Fe, quienes se empeñaron en enseñarme y dedicar su tiempo a formarme como médico y como persona; ellos fueron quienes me transmitieron la ilusión por esta especialidad, y quienes supervisaron mis primeros pasos en el camino a esta tesis; sin ellos no habría sido posible llegar hasta aquí.

A Yves Benoit, Matthias Karrasch y Stefan Suciu, mis mentores en la EORTC, quienes con su amabilidad e infinita paciencia, su sabiduría y su confianza, han hecho realidad la propuesta que hoy queda plasmada en estas páginas. A la fundación Schroeder que financió parte de mi beca en Bruselas.

A Birgit Geoerger, mi mentora en París, de quien he aprendido a ser riguroso en mi razonamiento científico, exigente y comprometido, exquisito en los detalles, y quien ha sabido hacerme progresar en la investigación y el desarrollo de nuevos medicamentos contra el cáncer.

A Luis Madero, mi jefe en el Niño Jesús, por haber confiado en mí desde el principio, por haber dedicado el poco tiempo que le permiten sus obligaciones a esta tesis, y motivarme para llevarla a cabo, ya que sin ese estímulo no habría sido posible hacerlo.

A Lucas. Mi residente mayor, mi compañero, y sobre todo mi amigo. Una persona a la que admiro, por ser brillante y humilde, por ser inteligente y amable, y con quien espero poder compartir muchos buenos momentos ahora que somos parte del mismo equipo.

A mis compañeros de oncología del Niño Jesús, quienes me han acogido con cariño y me han hecho fácil integrarme en su mundo, y hacen que cada día vaya feliz a trabajar.

A mis amigos, quienes dicen que algún día ganaré un Nobel (es una broma que hacen con frecuencia). A todos ellos, porque forman parte de esta historia, por estar siempre a mi lado cuando les he necesitado, y por haber compartido con ellos algunos de los mejores instantes de mi vida.

Y por supuesto a los niños y sus familias que han participado en estos estudios.

Gracias a todos.

INDEX

ACKNOWLEDGEMENTS..…….. 7
GLOSSARY…… 15
SUMMARY (English)…….…….…. 21
RESUMEN (Español)…….…….…. 24
I. INTRODUCTION…….…….. 27
1. Overview of cancer during childhood and adolescence…….… 29
2. Overview of leukemia during childhood and adolescence…….… 31
2.1. Acute lymphoblastic leukemia in children and adolescents…….… 31
2.2. Treatment of acute lymphoblastic leukemia…….…… 32
2.3. Complications of acute lymphoblastic leukemia treatment…….… 36
2.4. Treatment related deaths in children with acute lymphoblastic leukemia: A perspective…….……... 39
3. European organization for research and treatment of cancer (EORTC)…...… 42
3.1. EORTC aims and mission…….……..……... 42
3.2. EORTC Children's Leukemia group…….…… 43
II. HYPOTHESIS AND OBJECTIVES..…….…….. 47
1. Hypothesis…….……..……. 49
2. Objectives (English)…….……..…….. 50
3. Objetivos (Spanish)….…….……..…….. 51
III. PATIENTS AND METHODS……...…….…….. 53
1. Patients and methods…...…….……..……. 55
1.1. Data collection and evaluation of results…...…….……..….. 55
1.2. Selection of patients…...…….……..…… 55
1.3. Study group definitions…...…….……..…….. 55
1.4. National Cancer Institute (NCI) group definitions…… 58
1.5. Age/Time definitions……. 58
1.6. Disease definitions……. 59
1.7. Response criteria…...…….……..……. 61
1.8. Treatment guidelines and follow up…… 62
1.9. Ethical considerations and patient informed consent…… 62
1.10. Statistical analysis……..……. 63
1.11. Literature search strategy and bibliography…….……... 65

2. Protocols…… 65
2.1. Protocol 58741…… 65
2.2. Protocols 58831/58832…… 71
2.3. Protocol 58881…… 77
2.4. Protocol 58951…… 85
IV. RESULTS…….. 95
1. Results…… 97
1.1. Protocol 58741…… 97
1.2. Protocols 58831/58832…… 102
1.3. Protocol 58881…… 110
1.4. Protocol 58951…… 118
1.5. Summary of results…… 122
1.5.1. Early death…… 123
1.5.2. Death in first complete remission…… 127
V. DISCUSSION…….. 133
1. Discussion……. 135
1.1. Treatment related deaths in pediatric acute lymphoblastic leukemia……. 136
1.2. Early death…… 136
1.3. Death in first complete remission…… 139
1.4. Definition of treatment related mortality…… 142
VI. CONCLUSIONS..... 145
1. Conclusions (English)…… 147
2. Conclusiones (Español)…… 150
VII. REFERENCES..... 153
1. List of references……. 155
VIII. APPENDIX…….. 163
1. Description of treatment related deaths reported by other groups…… 165
2. Detailed description of the patients who died of toxicity on each EORTC protocol…… 173
3. Cumulative doses of chemotherapy of the EORTC-CLG ALL protocols……. 175

GLOSSARY

- **ADR:** Adriamycin (Doxorubicin)
- **AE:** Adverse event
- **AIEOP:** Associazione Italiana di Ematologia ed Oncologia Pediatrica
- **ALL:** Acute lymphoblastic leukemia
- **AML:** Acute myeloid leukemia
- **AR:** Average risk
- **ARA-C:** Cytarabine, Cytosine arabinoside, Aracytine
- **ARDS:** Adult respiratory distress syndrome
- **AUL:** Acute undifferentiated leukemia
- **BCG:** Bacillus Calmette-Guerin
- **BCNU:** Bis-chlorethyl-nitrosureum
- **BFM:** Berlin-Frankfurt-Munich group
- **BM:** Bone marrow
- **CB:** Cord blood
- **CCG:** Children's cancer group
- **CI:** Confidence interval
- **CLG:** Children's leukemia group
- **CMV:** Cytomegalovirus
- **CNS:** Central nervous system
- **COALL:** German Co-operative study group for treatment of acute lymphoblastic leukemia in childhood
- **COPAD:** Cyclophosphamide, vincristine, prednisolone and doxorubicin.
- **CPH:** Group for Pediatric Hematology in Czech Republic
- **CPM:** Cyclophosphamide
- **CR:** Complete remission
- **CRF:** Case report form
- **CSF:** Cerebrospinal fluid
- **CSI:** Craniospinal irradiation
- **CVC:** Central venous catheter
- **DBT:** Death before treatment
- **DCOG:** Dutch Childhood Oncology Group
- **DCR:** Death in first complete remission
- **DI:** DNA index
- **DIC:** Disseminated intravascular coagulation
- **DF:** Degrees of freedom
- **DFCI:** Dana-Farber Cancer Institute
- **DFS:** Disease free survival
- **DMSO:** Dimethylsulfoxide
- **DNA:** Deoxyribonucleic acid
- **DNR:** Daunorubicin
- **DS:** Down syndrome
- **DXM:** Dexamethasone
- **EBV:** Epstein Barr virus
- **ECHO virus:** Enteric Cytopathic Human Orphan virus
- **ED:** Early death
- **EFS:** Event free survival
- **EG:** Exempli gratia
- **EORTC:** European organization for research and treatment of cancer
- **EU:** European Union
- **FAB:** French-American-British morphology classification
- **G-CSF:** Granulocyte colony stimulating factor
- **GPR:** Good partial response
- **GVHD:** Graft versus host disease
- **HBV:** Hepatitis B virus
- **HD-MTX:** High doses methotrexate
- **HLA:** Human leukocyte antigen
- **HLH:** Hemophagocytic lymphohistiocytosis
- **HR:** High-risk
- **HSCT:** Hematopoietic stem cell transplantation
- **HSV:** Herpes simplex virus
- **ID:** Intradermally
- **IE:** Id est
- **IM:** Intramuscularly
- **INS:** Israel National Studies
- **IR:** Intermediate risk
- **IT:** Intrathecal therapy
- **IU:** International units
- **IV:** Intravenous
- **JCCLSG:** Japanese Childhood Cancer and Leukemia Study Group
- **LR:** Low risk

- **M:** Methotrexate based consolidation (EORTC-CLG Protocol 58741)
- **MAS:** Macrophagic activation syndrome
- **MDS:** Myelodysplastic syndrome(s)
- **MLL:** Mixed lineage leukemia
- **MMUD:** Mismatched unrelated donor
- **MMRD:** Mismatched related donor
- **MOF:** Multiorgan failure
- **MR:** Medium risk
- **MRC:** Medical Research Council
- **MRD:** Minimal residual disease
- **MSD:** Matched sibling donor
- **MTX:** Methotrexate
- **MTZ:** Mitoxantrone
- **MUD:** Matched unrelated donor
- **NCI:** National Cancer Institute
- **NHL:** Non-Hodgkin lymphoma
- **NK:** Not known
- **NOPHO:** Nordic society of paediatric haematology and oncology
- **NRM:** Non-relapse mortality
- **OR:** Odds ratio
- **OS:** Overall survival
- **P:** Polychemotherapy based consolidation (EORTC-CLG Protocol 58741)
- **PB:** Peripheral blood
- **PDN:** Prednisolone/Prednisone
- **PE:** Pulmonary embolism
- **PNET:** Primitive neuroectodermal tumor
- **PO:** Per oral
- **POG:** Pediatric Oncology Group
- **PR:** Partial remission
- **PTLD:** Post transplant lymphoproliferative disease
- **REAL:** Revised European-American classification of lymphoid neoplasms
- **RBC(s):** Red blood cell(s)
- **RF:** Risk factor
- **SE:** Standard error
- **SMN:** Secondary malignant neoplasm
- **SR:** Standard risk
- **SVC:** Superior vena cava syndrome
- **SVT:** Supraventricular tachycardia
- **TBI:** Total body irradiation
- **TCCSG:** Tokyo Children's Cancer Study Group
- **TLS:** Tumor lysis syndrome
- **TLP:** Traumatic lumbar puncture
- **TPOG:** Taiwan Pediatric Oncology Group
- **TRD:** Treatment related death(s)
- **TRM:** Transplant related mortality
- **VANDA:** Acronym for an ALL therapy including DXM, Ara-C, MTZ, VP-16, Asparaginase and IT chemotherapy
- **VCR:** Vincristine
- **VDS:** Vindesine
- **VHR:** Very high-risk
- **VLR:** Very low risk
- **VM-26:** Teniposide
- **VOD:** Veno-occlusive disease
- **VP-16:** Etoposide
- **VZV:** Virus varicella zoster
- **WBC:** White blood cell(s)
- **6-MP:** 6-Mercaptopurine
- **6-TG**: 6-Thioguanin

SUMMARY

SUMMARY

INTRODUCTION: Acute lymphoblastic leukemia (ALL) is the most frequent cancer type in children. Complete remission is achieved in more than 95% of patients and the five-year survival rate exceeds 90% in the developed countries with current protocols. Nevertheless, up to 5% of children die due to toxic side effects of anti-leukemic therapy in first line protocols. This study is focused on the treatment related deaths (TRD) occurring in pediatric patients with ALL treated in frontline trials from the EORTC Children Leukemia Group (EORT-CLG). This study evaluates the incidence and type of TRD and investigates the risk factors for increased likelihood of TRD in ALL pediatric patients.

PATIENTS AND METHODS: Retrospective study of patients included in the four EORTC-CLG trials for newly diagnosed ALL pediatric patients since 1971 to 2008. Treatment related deaths were categorized in:

- **Early death or death before first complete remission:** Patients who died after registration in the trial and before achieving a first complete remission following pre-phase and induction/consolidation therapies.

- **Death in first complete remission:** Patients who died in first CR for other reasons than ALL during or after having completed first line ALL-CLG therapies.

Ultimate cause of death was categorized in: tumor burden related deaths, infection, bleeding/thrombosis, organ toxicity not induced by infection, secondary malignancies, transplant related mortality, ALL progression and others. For the analysis of risk factors for death before remission and death in complete remission a multivariate analysis via logistic regression analysis was performed. Probabilities of survival were estimated using the Kaplan-Meier method. *P* values were statistically significant if <0.05.

RESULTS: In the analysis 4916 patients were included from four trials: 58741, 58831/2, 58881 and 58951 conducted in the decades of the 70´s, 80´s, 90´s and 2000´s respectively. The 58741 trial only included patients once in first remission. Incidence of treatment related deaths has significantly progressively reduced since first trial (p<0.001):

- **All treatment related deaths:** All trials (4.5%). By protocol: 58741 (8.1%), 58831/2 (3.4%), 58881 (3.9%) and 58951 (2.5%).

- **Early death:** All trials (1%). By protocol: 58831/2 (2.2%), 58881 (0.9%) and 58951 (0.7%).
- **Death in first complete remission:** All trials (3.5%). By protocol: 58741 (8.1%), 58831/2 (1.2%), 58881 (3%) and 58951 (1.8%).

Most frequent causes of death before remission were infection, bleeding and thrombosis and tumor burden related deaths. Bacterial organism mainly represents infections related deaths across all trials. In the last trial (58951), no patients died before any anti-leukemia treatment was initiated and no tumor burden related deaths were reported. The incidence of bleeding/thrombosis related death has progressively reduced. In the multivariate analysis age less than one year, female gender, NCI-High Risk and having been treated in the 58831/2 protocols were significantly associated with an increased risk of dying before remission.

Most frequent causes of death in first remission were infection, transplant related toxicities and secondary malignant neoplasms (SMNs). *Pneumocystis jirovecii* was a major cause of death in the 58741 before the introduction of systematic prophylaxis. Measles was the most frequent viral organism inducing deaths in the 58831/2 and 58881 trials, before implementation of widespread MMR vaccination. Transplant related mortality has decreased progressively in the last three trials (22%, 26% and 7%). SMNs are represented by acute myeloid leukemia and sarcoma type induced tumors. In the multivariate analysis age above ten years, having been transplanted in first remission and having been treated in the 58741 trial were significantly associated with an increased risk of dying in remission.

CONCLUSIONS: The progressive reduction in TRD is associated to:

- Improvement in supportive care measures
- Better recognition of patients with high-risk features for experiencing toxic events
- Progressive learning in treating institutions with similar protocols
- Better selection of patients candidates for transplantation in first remission
- Increased awareness and early recognition of therapy-induced toxicity
- Implementation of guidelines to prevent and minimize the toxic side effects of the chemotherapeutics used.

Patients with the characteristics identified in the multivariate analysis should be carefully monitored in order to identify early signs of toxicity that may prevent them of experiencing fatal

events. The incidence and type of treatment related deaths is similar to that reported from other collaborative groups in contemporary clinical trials and has evolved over the last forty years.

RESUMEN

INTRODUCCIÓN: La leucemia aguda linfoblástica (LLA) es el cáncer más frecuente en niños. Las tasas de remisión completa alcanzan el 95% y la supervivencia global a los 5 años supera el 90% en los países desarrollados con protocolos contemporáneos. Sin embargo, hasta un 5% de los pacientes fallecen por causas relacionadas con el tratamiento. El objeto de este estudio es la mortalidad relacionada con el tratamiento (MRT) en pacientes con LLA tratados en protocolos de primera línea del grupo pediátrico de la EORTC (EORT-CLG). Este estudio evalúa la incidencia y el tipo de la MRT e investiga los factores de riesgo para fallecer de muerte tóxica en pacientes pediátricos con LLA.

PACIENTES Y MÉTODOS: Estudio retrospectivo de los pacientes incluidos en los cuatro protocolos del grupo EORT-CLG para pacientes de nuevo diagnóstico de LLA desde 1971 a 2008. La mortalidad relacionada con el tratamiento se definió como:

- Muerte precoz o muerte antes de la primera remisión completa: Pacientes que fallecieron entre el momento del registro en el ensayo y antes de haber alcanzado una primera remisión completa tras la pre-fase, inducción y consolidación

- Muerte en primera remisión completa: Pacientes que fallecieron en primera remisión completa por otras causas diferentes a progresión de la LLA durante o después de haber completado el tratamiento de primera línea.

La causa última de muerte se categorizó como: mortalidad relacionada con la carga tumoral, sangrado/trombosis, toxicidad orgánica no inducida por infección, segundas neoplasias, toxicidad relacionada con el trasplante, progresión de LLA y otras. Para el análisis de los factores de riesgo antes de la primera remisión y en primera remisión se realizó un análisis multi-variante vía regresión logística. Las probabilidades de supervivencia se estimaron usando el método de Kaplan-Meier. Los valores *p* se consideraron estadísticamente significativos cuando fueron <0,05.

RESULTADOS: En el análisis se incluyeron 4916 pacientes de 4 ensayos clínicos: 58741, 58831/2, 58881 y 58951 llevados a cabo en las décadas de los 70, 80, 90 y 2000 respectivamente. El ensayo 58741 solo incluyó a pacientes una vez alcanzada la primera remisión. La incidencia de mortalidad relacionada con el tratamiento ha disminuido de forma progresiva y significativa desde el primer ensayo (p<0,001):

- **Todas las muertes relacionadas con el tratamiento:** Todos los ensayos (4,5%). Por protocolo: 58741 (8,1%), 58831/2 (3,4%), 58881 (3,9%) y 58951 (2,5%).
- **Muerte precoz:** Todos los ensayos (1%). Por protocolo: 58831/2 (2,2%), 58881 (0,9%) y 58951 (0,7%).
- **Muerte en primera remisión completa:** Todos los ensayos (3,5%). Por protocolo: 58741 (8,1%), 58831/2 (1,2%), 58881 (3%) y 58951 (1,8%).

Antes de la primera remisión, las causas más frecuentes de muerte fueron infecciones, sangrado y trombosis y muerte relacionada con la carga tumoral. Las bacterias fueron el microorganismo más frecuente entre las infecciones en todos los protocolos. En el último protocolo (58951) ningún paciente falleció antes de que se iniciase el tratamiento para la enfermedad y no hubo muertes relacionadas con la carga tumoral. La incidencia de causas relacionadas con sangrado o trombosis se ha reducido progresivamente. El análisis multi-variante mostró que los pacientes menores de un año, de sexo femenino, del grupo de alto riesgo NCI y aquellos que fueron tratados en los protocolos 58831/2 tenían mayor riesgo de mortalidad precoz.

En primera remisión completa, las causas más frecuentes de muerte fueron infecciones, toxicidad relacionada con el trasplante y segundas neoplasias. La infección por *Pneumocystis jirovecii* fue una causa mayor de mortalidad en el protocolo 58741 previo a la introducción sistemática de la profilaxis. El sarampión fue el virus más relacionado con mortalidad tóxica antes de la implementación de la vacunación universal en los protocolos 58831/2 y 58881. La mortalidad relacionada con el trasplante se ha reducido progresivamente en los últimos tres protocolos (22%, 26% y 7%). Las segundas neoplasias más frecuentes son leucemia mieloide aguda y sarcomas. El análisis multi-variante mostró que los pacientes mayores de 10 años, los trasplantados en primera remisión y aquellos tratados en el protocolo 58741 tenían mayor riesgo de mortalidad en remisión.

CONCLUSIONES: La progresiva disminución de la MRT está asociada a:

- Mejoría del tratamiento de soporte
- Mejor identificación de los pacientes con características de alto riesgo para experimentar mayor toxicidad
- Aprendizaje progresivo de las instituciones con protocolos similares
- Mejor selección de los pacientes candidatos a trasplante

- Mejor identificación de signos precoces de toxicidad inducida por el tratamiento e implementación de guías para prevenir y minimizar sus efectos secundarios.

Los pacientes con las características identificadas en el análisis multi-variante deben ser monitorizados y vigilados estrechamente con el objetivo de identificar signos precoces de toxicidad que permitan prevenir el desarrollo de eventos fatales. La incidencia y el tipo de MRT es similar a la descrita por otros grupos colaborativos en ensayos clínicos contemporáneos y ha evolucionado y cambiado a lo largo de estas cuatro décadas.

I. INTRODUCTION

1. OVERVIEW OF CANCER DURING CHILDHOOD AND ADOLESCENCE

The term childhood cancer usually comprises all cancers arising in individuals before the age of 15 years[1]. Histologically, childhood tumors are very variable and are classified into twelve major groups[1] (Figure 1), further divided into 47 diagnostic subgroups according to the International Classification of Childhood Cancer[2].

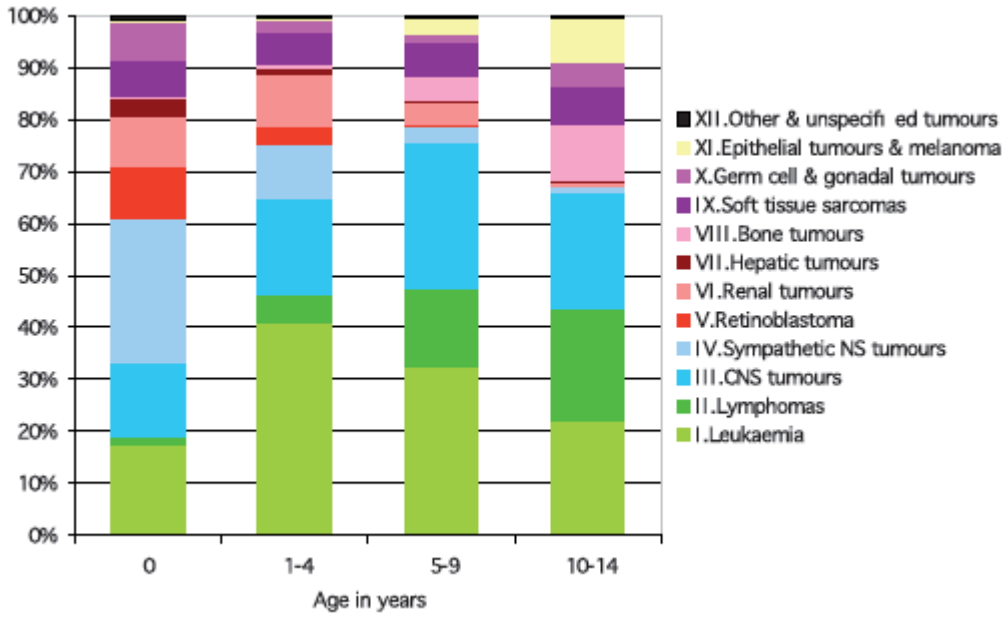


Figure 1. Composition of tumor types across childhood age groups[1].

In childhood populations of Europe, North America and other developed regions of the world, cancer incidence rates are around 140 per million. Cancer incidence in the developing countries is less well known, because there have been too few efficient population-based cancer registries. In some developing countries, where the children comprise 40–50% of the population, the proportion of childhood cancers represents 3–10% of the total, whereas in the developed countries, it is less than 1%. Mortality patterns also differ. Cancer accounts for some 4–5% of childhood deaths in developed countries, (where it is the second-leading cause of death among children aged 1–14), and less than 1% in developing countries, where deaths from infectious diseases are much more prominent[1].

Cancer in adolescents comprises all cases occurring in individuals aged 15 to 19 years. In the populations of Caucasian descent, the most common cancers are lymphomas and carcinomas.

Other frequent cancer groups are central nervous system (CNS) tumors, germ cell tumors and sarcomas of bone and soft tissues, with slightly different ranks in males and females[1] (Figure 2).

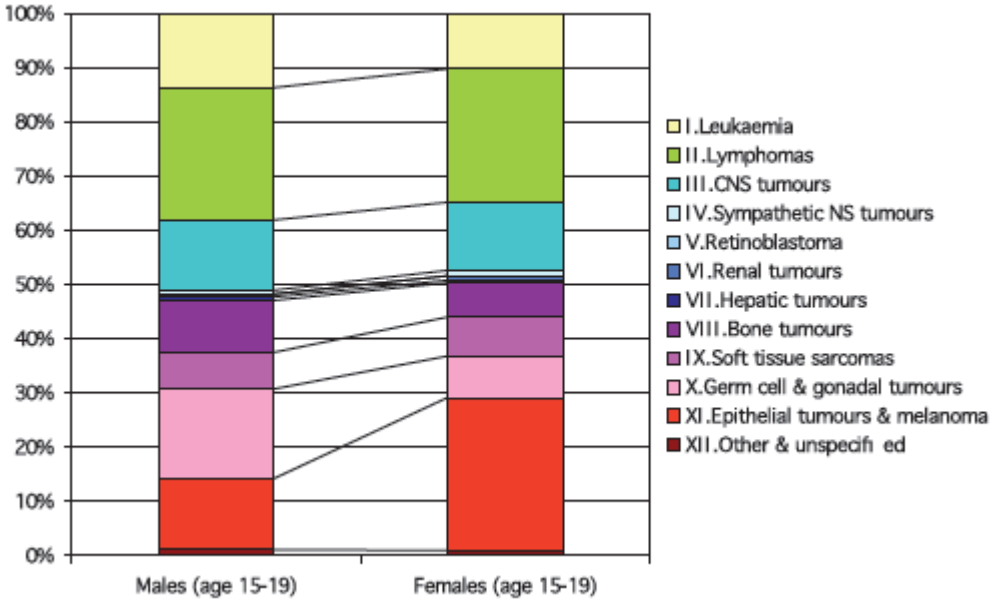


Figure 2. Composition of tumor types across adolescents[1].

Adolescence is the age of predominant occurrence of a few specific tumor types. Bone tumors (both osteosarcoma and Ewing tumor) usually present the first age-specific peak in adolescents overall and in males (in females the first peak of the two types of bone tumors occurs in the age group 10–14).

Survival probability has considerably changed within the past 30 years. Clinical research by cooperating groups of pediatric oncology centers has progressively increased the long-term survival rate from <20% before 1975 to >70-80% depending on the specific disease, in the new millennium[3].

International cooperation contributes to quality assurance because the majority of children with an oncologic disease are treated according to standard protocols. Reference centers therefore fulfill the important function of controlling, providing a second opinion and assessing the data of each child periodically[3].

2. OVERVIEW OF LEUKEMIA DURING CHILDHOOD AND ADOLESCENCE

The acute leukemias of childhood and adolescence are rare diseases than collectively represent around 30% of malignancies in children aged less than 14 years and 10% in those aged 15 to 19.

Leukemias can be classified as acute and chronic. *Acute leukemia* is characterized by clonal expansion of immature hematopoietic or lymphoid precursors, whereas *chronic leukemias* are characterized by expansion of mature marrow elements. This second group is very rare in children.

The acute leukemias are classified according to the predominant cell lineages involved, primarily either lymphoblastic or myelogenous. Approximately 80% of childhood acute leukemias are lymphoblastic (ALL) and 15% are myelogenous (AML). The remainder are difficult to characterize as either ALL or AML and are sometimes classified as undifferentiated (acute undifferentiated leukemia)[4].

2.1 Acute lymphoblastic leukemia in children and adolescents

ALL is the most frequent cancer type in children. Thirty-eight out of one million children are newly diagnosed with ALL annually. It is more frequent in boys than in girls (2:1 ratio) with a peak incidence between 2 and 5 years of age. The incidence in white children is twice as high as in non-white children.

The history and symptoms reflect the degree of bone marrow infiltration by leukemic cells and the extramedullary involvement of the disease. The disease can affect almost any organ. The duration of symptoms may be days to several weeks, and occasionally months.

Abnormal laboratory findings are commonly seen at the time of presentation. The number of red blood cells (RBC), platelets and white blood cells (WBC) may vary depending on the degree of bone marrow involvement. Associated electrolyte disturbances are frequently observed as a reflect of the compromise of organ function due to infiltrating blast cells. An appropriate management of these alterations is crucial at the very beginning of the diagnosis and treatment, as it will be discussed later.

The diagnosis of ALL is based most of the times on the findings from the bone marrow. Nevertheless, sometimes the diagnosis can be established by means of the results obtained from blood or other biological samples.

The leukemic characterization and classification is made based on the morphology (FAB classification[5]), cytochemistry, immunology, flow cytometry, biochemistry, cytogenetic and molecular aspects of the malignant cells. Based on the patterns of reactivity with a panel of lineage-associated antibodies, ALL has been sub classified into three broad categories: mature B-cell ALL, B-progenitor ALL and T-progenitor ALL. In some instances, individual leukemic cells simultaneously express both lymphoid and myeloid surface antigens. These leukemias have been referred to as mixed lineage or biphenotypic leukemia. The immunophenotypic subsets are associated with distinctive clinical and laboratory features[3,4].

2.2 Treatment of acute lymphoblastic leukemia

With current regimens for the treatment of childhood ALL, complete remission is achieved in more than 95 per cent of patients and the five year survival rate exceeds 90% in the developed countries[6]. This is true despite the fact that, with few exceptions, the drugs used for the treatment of ALL today were available by the late 1960s[7–9]. This improvement in outcome has been largely achieved as a result of international collaborative efforts through clinical trials and study groups[6].

The rate of success in the treatment of ALL has increased steadily since the 1960s[4,10]. Before 1947, when Farber and co-workers attained the first complete remission in a child with ALL, the median duration of survival from the time of diagnosis was 2 months. During the 1950s drugs such as 6-mercaptopurine (6-MP), methotrexate (MTX) and corticosteroids were found to be active in leukemia-bearing mice and subsequently in human leukemias. Some of the first controlled trials were conducted at that time, consisting of single-agent antileukemic chemotherapy protocols. Active drugs introduced in the 1960s and 1970s included the anthracyclines (doxorubicin and daunorubicin), asparaginase and the epipodophyllotoxines (etoposide [VP-16] and teniposide [VM-26]).

To identify treatment components responsible for improved treatment outcome, the Childhood ALL Collaborative Group was formed in 1994 to systematically review and analyze results from relevant randomized trials. Analysis of data from four clinical trials that enrolled patients between 1972 and 1984 showed that anthracyclines reduced hematologic relapse but failed to improve event-free survival, partly because of the increased induction failures and deaths in remission[11]. Meta-analysis of trials started between 1965 and 1998 showed that the

addition of vincristine plus prednisone or prednisolone pulses during post-remission therapy improved event-free survival; the lack of improvement with vincristine and dexamethasone pulses in the more recent trials was attributed to the greater intensity of the early therapy[12]. Meta-analysis of three trials that enrolled patients between 1992 and 2002 suggested that thioguanine improved event-free survival compared with mercaptopurine for males younger tan age 10 years, but its lack of effect on survival and association with a high risk of veno-occlusive disease (VOD) of the liver made mercaptopurine the standard thiopurine of choice[13]. Meta-analysis of 47 trials of CNS-directed therapy conducted between 1970 and 1999 showed that CNS radiotherapy can generally be replaced by intrathecal therapy, and triple intrathecal therapy should be used with effective systemic therapy such as intravenous high-dose methotrexate to realize its full benefit of CNS control without the hazard of increased systemic relapse[14].

Besides this, significant efforts have been made to harmonize definitions in ALL, so that investigators around the world could use the same terminology. In this sense, participants in *The Ponte di Legno* group meeting in 1995 agreed to use similar criteria to present the treatment outcome so that results could be compared among various clinical trials to identify effective treatment strategies for specific subsets of ALL[15,16].

Regarding the biology of ALL in specific group of patients, big advances have been made in the last few years. Some examples are depicted in table 1[6]:

Table 1. Clinical research findings from selected collaborative studies					
Subgroup of ALL	**Years Study**	**Number Study Groups**	**Number Patients**	**Major Findings**	**Reference**
***11q23* rearranged**	1983-1995	11	497	Prognosis was particularly dismal in infants; allogeneic transplantation did not improve outcome in patients with *t(4;11)* ALL. Poor early response to prednisone or age younger than 3 months conferred a particularly dismal prognosis among infants with *t(4;11)*.	17,18
Hypodiploid	1986 1996	11	139	Prognosis was very poor, especially among patients with fewer than 44 chromosomes.	19
Hypodiploid	2008 2013	2	126	Genome profiling and sequencing identifies distinct genetic alterations in subtypes of hypodiploid ALL. Near-haploid patients have Ras pathway mutations and *IKZF3* mutations; low-hypodiploid patients have *IKZF2* alterations and *TP53* mutations, many of which are inherited.	20
Ph positive	1986 1996	10	326	Presenting age, leukocyte counts, and response to initial treatment with glucocorticoids and intrathecal methotrexate affected treatment outcome; matched-related transplantation improved outcome.	21
Ph positive	1995 2005	10	610	Both matched-related and matched-unrelated transplantation improved treatment outcome.	22
Ph positive	2004 2009	10	178	Imatinib combined with intensive chemotherapy was well tolerated and might improve outcome.	23
Ph-like	2008 2009	2	221	Genetic alteration of *IKZF1* was associated with high levels of minimal residual disease and a very poor outcome.	24
Ph-like	2008 2009	2	297	These cases were associated with a high frequency of deletions in genes involved in B-cell development, resistance to asparaginase and daunorubicin, and unfavorable outcome.	25
Ph-like	2014	5	264	More than 90% of the patients had kinase-activating alterations, some of which were amenable to inhibition with tyrosine kinase inhibitors such as imatinib or dasatinib.	26
Early T-cell precursor	1992 2006	2	30	These patients had distinctive immunophenotype (CD1a, CD8, or CD5 weak with stem-cell or myeloid markers) and high risk of remission induction failure or hematologic relapse.	27
Down syndrome	1995 2004	16	653	Compared with other patients, these patients have lower event-free survival as a result of increased relapse hazard and treatment-related mortality. Younger age and leukocyte count $<10 \times 10^9$/L at diagnosis and the presence of high hyperdiploidy and *ETV6-RUNX1* are favorable prognostic factors.	28
Induction failure	1985 2000	14	1041	Patients with induction failure are highly heterogeneous. Although allogeneic transplantation improved outcome for T-cell ALL, chemotherapy should be the treatment of choice for B-ALL without other adverse features.	29

Summarizing, the following factors have been identified to have an impact in outcome:

- The development of complex chemotherapeutic regimens designed to achieve clonal eradication.
- The development of cell kinetic studies that have been important in the design of timed sequential use of cyto-toxic drugs.
- Biology studies that have permitted to identify particular molecular and genetic alterations enabling physicians to identify patients at higher risk of relapse and therefore adapting therapy according.
- The recognition of the central nervous system as a sanctuary site that has permitted to adapt therapy evolving from craniospinal radiotherapy to systemic and intrathecal chemotherapy.
- The improvements in supportive care.
- Clarification of risk groups. Classical classification into standard, intermediate and high-risk groups is based on age, white blood cell counts at diagnosis, extent of disease (CNS or testicular involvement), response to chemotherapy after pre-phase and at the end of induction and biological findings.

In general, current treatment regimens for patients with newly diagnosed ALL include four phases:

- **Remission induction:** Designed to rapidly destroy measurable leukemic cells and minimize residual leukemic burden (i.e. the total number of leukemic cells in the body).
- **CNS-directed therapy:** Used to address the issue of pharmacological sanctuary sites (i.e. areas of the body, such as the brain and spinal cord, that are not well penetrated by conventional doses of most antileukemic drugs).
- **The intensification/consolidation phase:** Designed to further reduce the total body leukemia cell burden and address issues of antileukemic drug resistance. Such treatment usually consists of higher doses of the same drugs used during induction or of high doses of different drugs.
- **The maintenance/continuation phase:** Considered to eradicate the residual leukemic cell burden and avoid relapse.

Attempts to boost cure rates further with the use of hematopoietic stem cell transplantation (HSCT) have improved the outcome for some, but not all, subtypes of ALL, suggesting that intensification of existing treatments is unlikely to rise cure rates substantially and will instead increase treatment related toxicity and the risk of such life-threatening late sequelae as second cancers.

2.3 Complications of acute lymphoblastic leukemia treatment

At the time of diagnosis, the major clinical problems arise from metabolic disturbances secondary to leukemia cell lysis, disrupted hematopoiesis leading to abnormal peripheral blood counts, and leukemic infiltration of non-hematopoietic organs. Initiation of chemotherapy may exacerbate these issues and lead to additional problems, including myelosuppression, increased risk of infections and mucosal toxicity.

Complications of treatment will be described in this section with more detail as they are the subject of this study[4,30]:

- **Metabolic complications:** When effective chemotherapy was first employed in acute leukemia, rapid lysis of leukemic cells often resulted in serious and occasionally fatal metabolic disturbances (hyperkalemia, hyperuricemia, hyperphosphatemia and hypocalcemia). This is particularly worrying in situations with high burden of disease (e.g. white cell count exceeds $100x10^9$/l (10 to 20 percent of acute leukemia)). The introductions of allopurinol, and more recently recombinant urate oxidase (rasburicase), together with a skill fluid electrolyte therapy, have done much to solve this problem[4,30].
- **Anterior mediastinal mass:** Leukemic infiltration of the thymus gland appears as an anterior mediastinal mass on chest radiography. It is observed in about 10 percent of patients with newly diagnosed ALL and is nearly always associated with the T-cell immunophenotype. Leukemic infiltration of the mediastinal structures may cause life-threatening tracheobronchial or cardiovascular compression leading to a superior vena cava syndrome (SVC). Pleural effusion may also be associated. Prompt initiation of systemic chemotherapy (such as corticosteroids) is necessary to handle such emergencies and rarely emergency radiation therapy may be necessary[4,30].

- **Anemia and thrombocytopenia:** Thrombocytopenia, in association with anemia, is a common presenting feature of ALL; however, active bleeding is a relatively unusual feature at the time of diagnosis. Any active hemorrhage associated with a platelet count less than $100x10^9$/l should be treated with platelet transfusion. Similarly, symptomatic anemia should be treated by transfusion of packed red blood cells. Stabilization of these two parameters should take no longer than 12 to 24 hours and should therefore not delay the start of antileukemic therapy[4,30].
- **Coagulation abnormalities:** Disseminated intravascular coagulation (DIC) is rarely observed in patients with ALL at diagnosis. It is most often seen in T-cell ALL as a result of thromboplastic substances released from the blasts as well as in patients with the uncommon t(17;19) translocation associated with the B-progenitor immunophenotype. L-asparaginase can induce a coagulopathy by inhibition of protein synthesis of clot forming and clot inhibitory proteins. CNS and peripheral thrombosis and hemorrhage have been observed in 1 to 2 percent of patients receiving asparaginase therapy[4,30].
- **Infection:** Infection due to granulocytopenia is an important early complication of ALL, observed at the time of diagnosis and during therapy. Most infections are presumed to be bacterial, but specific etiologic agents often are not identified. Cultures should be obtained promptly, and the patients should immediately be given broad-spectrum intravenous antibiotics with activity against bowel and respiratory agents. Documented episodes of bacteremia have been reported in 15 to 30 percent of children receiving chemotherapy for ALL. As chemotherapy regimens have become more intensive, fungal disease, including invasive infections of *Candida* and *Aspergillus* species, has been increasingly observed during the initial treatment of leukemia. Viral infections, including varicella complicated by pneumonitis, hepatitis, or cerebritis can be particularly devastating. It became a major problem, especially with prednisone therapy, and many children died of severe disseminated varicella, while others had treatment interrupted for long periods with consequent risk of relapse. *Pneumocystis jirovecii* can cause a severe, often fatal, interstitial pneumonitis. The incidence of *P. Jirovecii* increases with the degree and duration of

immunosuppression and thus is usually observed during the later phases of ALL. Prophylaxis with trimethroprim-sulfamethoxazol markedly reduces its incidence[4,30].

- **Typhlitis:** This is neutropenic enterocolitis. It most commonly occurs in the caecum and typically follows a prolonged course of neutropenia, resulting in benign mucosal ulceration, bacterial invasion and bowel perforation and peritonitis. Management includes bowel rest and administration of intravenous fluids and broad-spectrum antibiotics. Surgery is usually not indicated except for the rare occurrence of perforation or uncontrolled bleeding[4,30].
- **Acute neurologic toxicity:** Seizures and other acute neurologic events have been observed in up to 20 percent of children with ALL. Cranial irradiation, intrathecal chemotherapy and high doses of systemic methotrexate, have been associated with acute neurotoxicity. Peripheral and autonomic neuropathy associated with vincristine is often seen in children receiving therapy for ALL[4,30].
- **Cardiac sequelae:** Echocardiographic abnormalities, in particular increased after load and decreased contractility, are common late effects of anthracycline therapy. The severity of cardiac dysfunction is correlated with higher cumulative doses of anthracycline and higher doses rate. Patients treated at a young age, females and those with Down syndrome appear to be more vulnerable to anthracycline-related cardiac toxicity[4,30].
- **Hematopoietic stem cell transplantation (HSCT) related complications:** HSCT has been used as part of the multimodal approach for the treatment of children with ALL. Although is mainly used in the context of relapsed ALL, HSCT has been considered in first complete remission in very selected groups of patients such as those harboring t(9;21) or high levels of minimal residual disease at the end of induction among others. Infectious complications constitute the major cause of morbidity and mortality in pediatric and adult patients given HSCT[31,32]. The incidence and the severity of infections strongly correlate with the use of immunosuppressive therapy and the time of neutrophil and immune reconstitution following HSCT. The risk of infection is higher in patients after allogeneic than autologous HSCT, in those with graft versus host disease (GVHD), and with delayed

immune reconstitution, especially following haploidentical and cord blood HSCT. Other complications include GVHD per se, veno-occlusive disease and specific organ toxicities (liver, kidney or lung). Outcome of patients undergoing HSCT has notably improved during the last years by a more precise selection of potential candidates along with a notably improvement in supporting measures adopted by the transplant units[33].

- **Second malignant neoplasms (SMN):** Second malignant neoplasms including malignant gliomas, AML and carcinomas of the parotid and thyroid glands, have been reported in survivors of ALL. Risk factors for the development of secondary AML include treatment with epipodophyllotoxines and alkylating agents [4,34,35]. Secondary solid tumors have been observed in patients who received cranial or craniospinal irradiation (CSI), especially those receiving higher doses (1800 to 2400 cGy). Long-term cumulative incidence rates of secondary neoplasms range between 1 and 3 per cent[4,36].
- **Central venous catheters related complications:** Indwelling central venous catheters (CVC) have been in use for the last three decades and have revolutionized the care of children with cancer and those requiring long-term access for medications and blood products. Central venous device–related complications have been extensively described in the literature. They can be approximately divided by thirds among infectious events, venous thrombosis, and mechanical events and up to 40 per cent of children with CVC will experience any of these complications[37–39].
- **Other toxicities:** Asparaginase is associated with pancreatitis in 5 to 10 percent of patients and allergic reactions in up to one third. Complications due to corticosteroids include hypertension, hyperglycemia, avascular necrosis, mood and behavioral problems and an increased incidence of bone fractures[4,30]. Inadvertent intrathecal administration has been reported with fatal outcome[40] so strict guidelines are to be implemented in ALL protocols to prevent such an unfortunate event[41].

2.4 Treatment related deaths in children with acute lymphoblastic leukemia: A perspective

Impressive advances in the management of childhood ALL in recent decades have resulted in constantly increasing survival rates of children and adolescents with ALL as

described above. A major drawback of this success history is the fact, that in most frontline multicenter trials of childhood ALL, up to 5% of children die due to toxic side effects of antileukemic treatment[8,42]. Besides that, some patients still die before any anticancer therapy can be started, suggesting a more aggressive behavior of the disease that also relates to the supportive care of children with leukemia given at this very early stage.

The majority of treatment related deaths (TRD) occur during recurring and prolonged episodes of neutropenia and lymphopenia caused by cytotoxic and immunosuppressive drugs or by the leukemia itself inhibiting bone marrow recovery during induction therapy. The rest of TRD relate to specific drug toxicities or supportive care complications, other than infections: chemotherapy causes immunosuppression; high-dose steroids cause immunosuppression as well as metabolic disturbances and the children often suffer from suboptimal nutrition; indwelling catheters, repeated admissions and the frequent and widespread use of antibiotics increases the risk of infections; the malignant disease itself may lead to hemorrhages, thrombosis, need of transfusions and tumor lysis complications before or after the initiation of therapy[8,43]; some drugs have specific toxicities that have been recognized as cause of death both during active treatment or after it (e.g. asparaginase induced pancreatitis, anthracycline related cardiotoxicity); radiotherapy has potential acute and delayed side effects adding toxicity to conventional concurrent chemotherapy[44,45]; in the recent years, HSCT has been implemented in first line therapies for children with special ALL features aiming to overcome the limitations of conventional chemotherapy but also adding specific toxicities in this group of patients[46].

Therefore, infections, bleeding or thrombosis, tumor burden complications and therapy induced organ toxicities are the most common causes of TRD. To summarize, four major factors influence the risk of these and other severe toxicities[42]:

- The leukemia itself (e.g. the tumor burden and specific organ involvement).
- Intensity of treatment.
- The supportive care (including specific guidelines and physician and patient compliance to these).
- Host factors (including inherited genetic polymorphisms that influence drug disposition and immune function).

A description of TRD reported by other groups[7–9,42,43,47–65] is shown in Appendix 1. In general, death rates occurring before remission (i.e. before any anticancer therapy is started or during induction) range between 0.3 and 3 per cent, while those occurring after first complete remission range between 1.5 and 5.3 per cent. A progressive reduction in TRD has been demonstrated by the majority of the groups along the years, reflecting a more accurate selection of patients by risk stratification factors and subsequently more directed therapy, an improvement in the supportive care measures and a learning process by a progressive implementation of protocols in the different cancer centers, among others.

Collaborative study groups have identified some factors that may put children at a higher risk of TRD. Age less than one year or more than ten, high leukocyte count at diagnosis ($>100x10^9$/L), female gender, T cell-immunophenotype and constitutional trisomy 21 are already reported[7,42,43]. Patients undergoing HSCT in first CR have been also considered as more prone to experience toxic deaths than those who receive conventional chemotherapy[42].

Most of these studies come from high-income countries where the degree of development and the availability of resources are much higher. For this reason, other groups have focused in the analysis of TRD in low-income countries. Unfortunately in these countries, the outstanding figures achieved in Europe, North America or some Asian countries are far less obtained. Potential reasons for different survival rates include a higher rate of relapse, more abandonment of treatment, and higher rates of TRD, even when similar or modified European or North American protocols are used. TRD rates in low-income countries using these protocols vary between 10 to 20 per cent[66–68]. It has been hypothesized that underlying population biological features as well as more advanced stage at presentation may change the underlying relationship between clinical parameters and TRD[69]. On the other hand, other investigators have found that socioeconomic factors like low income and lower parental education are bigger contributors to TRD than biological features[70].

Minor changes in chemotherapeutic treatment composition and scheduling have been repeatedly reported to have an impact on survival rates but also on treatment-related toxicity and mortality. Liang et al found that L-asparaginase used in the induction was associated to a higher degree of toxicity and toxic deaths when compared to an anthracycline based regimen[64]. Belgaumi et al reported that the addition of daunomycin to a dexamethasone based induction

regimen lead to a significant increase in the toxicity rates in children with B-precursor ALL[71]. These experiences highlight the difficulties of balancing the risk for fatal-related complications against the benefits that a different approach may have on the disease outcome.

The results presented here reflect the accumulated experience of the EORTC-CLG over 40 years in children ALL trials. This study will focus on the incidence, pattern and causes of death as first event and its predisposing factors for all eligible children enrolled in four consecutive trials.

3. EUROPEAN ORGANIZATION FOR RESEARCH AND TREATMENT OF CANCER (EORTC)

The European Organization for Research and Treatment of Cancer (EORTC) was founded as an international organization under Belgian law in 1962 by eminent oncologist working in the main cancer research institutes of the European Union (EU) countries and Switzerland. It was named the 'Groupe Européen de Chimiothérapie Anticancéreuse' and became EORTC in 1968 (www.eortc.org).

3.1 EORTC aims and mission

The aims of the EORTC are to develop, conduct, coordinate, and stimulate translational and clinical research in Europe to improve the management of cancer and related problems by increasing survival but also patient quality of life. Extensive and comprehensive research in this wide field is often beyond the means of individual European hospitals and can be best accomplished through the multidisciplinary multinational efforts of basic scientists and clinicians.

The ultimate goal of the EORTC is to improve the standard of cancer treatment through the testing of more effective therapeutic strategies based on drugs, surgery and/or radiotherapy that are already in use and also through the development of new drugs and other innovative approaches. This is accomplished mainly by conducting large, multicenter, prospective, randomized, phase III clinical trials. In this way, the EORTC facilitates the passage of experimental discoveries into state of the art treatments.

Through translational and clinical research, the EORTC offers an integrated approach to drug development, drug evaluation programs and medical practices.

EORTC Headquarters, a unique pan European clinical research infrastructure, is based in Brussels, Belgium, from where its various activities are coordinated and run. The EORTC is both multinational and multidisciplinary, and the EORTC Network comprises over 300 hospitals and cancer centers in over 30 countries, which include some 2,500 collaborators from all disciplines involved in cancer treatment and research.

The 180 members of the EORTC Headquarters staff handle some 6,500 new patients enrolled each year in cancer clinical trials, approximately 30 protocols that are permanently open to patient entry, over 50,000 patients who are in follow-up, and a database of more than 180,000 patients.

Intergroup collaboration is also promoted to face current challenges of clinical trials aiming at targeted therapies.

3.2 EORTC Children's Leukemia group

The EORTC Children's Leukemia Group (CLG) is one of the 21 cooperative groups working within the structure of the EORTC. The CLG is part of the offspring of the EORTC Hemopathies Working Party which in 1978 split into a pediatric and to an adult branch.

At that time, the Berlin-Frankfurt-Munster (BFM) designed by H. Riehm for acute lymphoblastic leukemia appeared much more efficacious than all others and the CLG decided to adapt that treatment strategy for its own clinical trials.

Since then, the group has actively worked in the development of clinical trials in the field of malignant blood diseases in children including ALL, acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and lymphomas.

Some of the most relevant findings in the EORT-CLG trials are depicted in table 2, which exemplary the philosophy of this group, its contributions in the field of pediatric ALL and how different questions have been answered over time.

Currently the group is constituted by 24 centers spread over three countries: Belgium, France and Portugal.

The aims of the group are:

- To improve the treatment of leukemia and related malignancies (lymphomas, MDS) by means of clinical trials.

- To promote the study of these diseases with regard to their epidemiology and the long-term sequelae of treatment.
- To promote the clinical application, on a large number of patients, of new biologic techniques.

Table 2. Summary of the most relevant findings in the EORTC-CLG trials					
Name of study	**Years Study**	**Patients enrolled (n)**	**Patients**	**Major Findings**	**Ref**
58741	1971 1978	217	Children and adult with ALL in first CR	**A) 1st Randomization between 1 year polychemotherapy (P) or methotrexate (M) based consolidation** Consolidation P was more toxic than consolidation M. No differences in disease free interval o survival duration **B) 2nd Randomization between 4 year chemotherapy (C) or immunotherapy (I) based maintenance** Immunotherapy maintenance showed a superiority regarding chemotherapy maintenance	72
58831 **58832**	1983 1989	780	Children with ALL	**A) Patients with SR-ALL were randomized to receive cyclophosphamide (CPM) (1 g/m^2 days 1 and 29 phase IB):** Absence of CPM had no impact on outcome: 10 years DFS rates ±1 s.e. 71% ± 3.3% and 70% ± 3.7% with and without **B) Medium and HR patients were randomized to receive cranial radiation after protocol II containing HD-MTX:** HD-MTX alone was as effective as HD-MTX followed by cranial radiotherapy in terms of DFS, isolated CNS relapse-free interval and CNS relapse-free interval	44
58881	1989 1998	2078	Children with ALL	**A) Patients were randomized to receive high doses cytarabine (1g/m^2) during interval therapy:** the combination of cytarabine at high doses with methotrexate at high doses during interval therapy did not improve prognosis B) **Patients were randomize to test the advantage of adding monthly intravenous 6-mercaptopurine (1 g/m^2) to conventional maintenance:** The addition of 6-mercaptopurine iv during maintenance increased the risk of late relapse **C) Patients were randomized to evaluate the efficacy and the toxicity of Erwinia asparaginase vs E. coli asparaginase:** E. coli asparaginase was more toxic but has a higher efficacy than Erwinia asparaginase. EFS rates at 5 years were 74.0% ± 2.4% in the E. coli group vs 61.1% ± 2.7% in the Erwiniase group. Survival rates at 5 years were 83.2% ± 2.0% in the E. Coli group vs 76.8% ± 2.4% in the Erwiniase group.	44
58881	1989 1998	178	Children with ALL	The presence or absence and level of residual leukemia were significantly correlated with the risk of early relapse at each of the times studied (P<0.001). Residual leukemia after induction of a remission is a powerful prognostic factor in childhood ALL.	73
58881 **58951**	1989 2008	134	Children with T-ALL	NOTCH+ (NOTCH1 and/or FBXW7 mutated)) patients showed a better early response to chemotherapy as compared with NOTCH- patients. 5-year EFS of 73% and 70% (P=0.82), and 5-year OS of 82% and 79% (P=0.62) for NOTCH+ and NOTCTH-.	74
58951	1998 2008	1947	Children with ALL	**A) Patients were randomized to dexamethasone (6 mg/m^2/day) to prednisolone (60 mg/m^2/day) in induction therapy:** The 8-year event-free survival rate was 81.5% in the dexamethasone arm and 81.2% in the prednisolone arm; the 8-year overall survival rates were 87.2% and 89.0% respectively. Incidence of G3-4 toxicities was similar. Dexamethasone decreased the 8-year central nervous system relapse incidence by 1.6%.	75
58951	1998 2008	1223	Children with ALL	Patients with IKZF1(del) had a lower 8-year event-free survival (EFS, 67.7% versus 86.5% (p<0.001). IKZF1(del) increased risk only in the high hyperdiploid ALLs and in 'B-other' ALLs, that is, lacking classifying genetic lesions.	76

ALL: Acute lymphoblastic leukemia; **CNS:** Central nervous system; **CPM:** Cyclophosphamide; **CR:** Complete remission; **DFS:** Disease free survival; **EFS:** Event free survival; **HD-MTX:** High doses methotrexate; **HR:** High-risk; **OS:** Overall survival.

II. HYPOTHESIS AND OBJECTIVES

1. HYPOTHESIS

Over the last decades we have assisted to a substantial increase in survival rates of children and adolescents with acute lymphoblastic leukemia (ALL). This improvement has been largely achieved as a result of the optimization in the use of existing antileukemic agents and the progressive introduction of new agents, better risk-group stratification, improvement in the knowledge of leukemia biology and supportive care in contemporary clinical trials. However, a number of children, who might otherwise be cured, still tragically die before they can be started on any anti leukemic treatment or from complications of the treatment itself.

Therefore, is of paramount importance to identify what factors may put some patients at risk of dying from treatment related complications. That could be achieved by an analysis of the long-term results of big cooperative groups using similar approaches along an expanded period of time. The Children's Leukemia Group from the European Organization for Research and Treatment of Cancer (EORTC-CLG), with a vast experience in the development of international collaborative protocols in childhood ALL, is in the position to face this challenge.

Changes in the strategies for patients with ALL over the last decades, such as modifications in the dose intensity, risk stratification and the use of bone marrow transplantation in selected cases might have had an impact in the incidence and type of treatment related deaths in children with newly diagnosed ALL.

The pattern of infectious and non-infectious complications may have evolved over time with the introduction of novel antimicrobial agents and improvement in supportive care.

We propose to analyze the impact of the different first line treatment protocols developed by the EORT-CLG on the type and number of treatment related deaths over the last forty years. We believe that the results obtained from this study will help us to increase and improve our understanding about treatment related toxicities. These results may help us in the future to adapt and individualize ALL therapies in order to minimize their undesirable side effects while maximizing their benefits.

2. OBJECTIVES

2.1 Primary objective

- To assess the types and causes of treatment related deaths in children with newly diagnosed ALL treated according to the first line EORTC-CLG ALL protocols 58741 – 58831/2, 58881 and 58951 from 1971 to 2008.

2.2 Secondary objectives

- To define the incidence and causes of treatment related deaths in the four trials grouped in two categories: early death (death before first complete remission) and death in first complete remission.
- To assess whether the treatment era carries an impact in the incidence of treatment related deaths.
- To identify risk factors of increased likelihood of treatment related deaths in children with ALL.
- To compare the incidence and type of treatment related deaths of the EORTC-CLG ALL protocols with the results reported by other collaborative groups.

3. OBJETIVOS

3.1 Objetivo primario

- Describir los tipos y causas de mortalidad relacionada con el tratamiento en niños con leucemia linfoblástica aguda de nuevo diagnóstico tratados en los protocolos de primera línea del grupo EORTC-CLG 58741, 58831/2, 58881 y 58951 desde 1971 a 2008.

3.2 Objetivos secundarios

- Definir la incidencia y causas de muerte relacionadas con el tratamiento en los cuatro ensayos clínicos agrupados en dos categorías: Muerte precoz o muerte antes de la primera remisión completa y Muerte en primera remisión completa.
- Averiguar si la era de tratamiento impacta en la incidencia de muertes relacionadas con el tratamiento.
- Identificar factores de riesgo para fallecer de muerte tóxica en pacientes pediátricos con LLA.
- Comparar la incidencia, el tipo y la causa de mortalidad relacionada con el tratamiento de los protocolos EORTC-CLG con los resultados publicados por otros grupos colaborativos.

III. PATIENTS AND METHODS

1. PATIENTS AND METHODS

1.1 Data collection and evaluation of results

All the data were collected, processed and analyzed at the EORTC data center in Brussels.

Data for this study was obtained from three different sources:

- Electronic databases for each of the corresponding trials that contain all the coded information about patients. Data extraction was made by means of two-computer software: Vista Update and Vista Stat.

- Patient´s files that contain the entire corresponding patient case report forms (CRFs).

- Queries to sites when data considered essential for this study could be retrieved neither from the electronic database nor from the patient´s files.

1.2 Selection of patients

The criteria to include patients in this study were:

- Age < 18 years at the time of diagnosis.
- Diagnosis of 'de novo' ALL not previously treated except for the reasons specified in each single protocol.
- To be eligible according to the inclusion/exclusion criteria specified in each of the EORTC ALL-CLG protocols.
- For those who completed induction, available data about response evaluation.

Patients who committed any sort of violation foresaw by the protocol along treatment and were therefore not kept in the trial for treatment purposes anymore, were kept nevertheless for the follow up and survival analysis purposes.

1.3 Study group definitions

Patients considered to be the subject of this study were included in two groups (Figure 3):

- **Early death (ED) included (Also named death before first complete remission):**
    - Death before treatment (DBT): Patients who died after having been registered into the trial and before any anticancer therapy was initiated.

- Death during prephase/induction/consolidation: Patients who died after having been registered into the trial and before achieving a first complete remission following pre-phase and induction/consolidation therapies.

- **Death in first complete remission (DCR):** Patients who died in first CR for other reasons than ALL during or after having completed first line ALL-CLG therapies. Patients who died of hematopoietic stem cell transplantation (HSCT) related toxicity performed in first CR, were included in this group and considered as cases of **transplant related mortality (TRM)**.

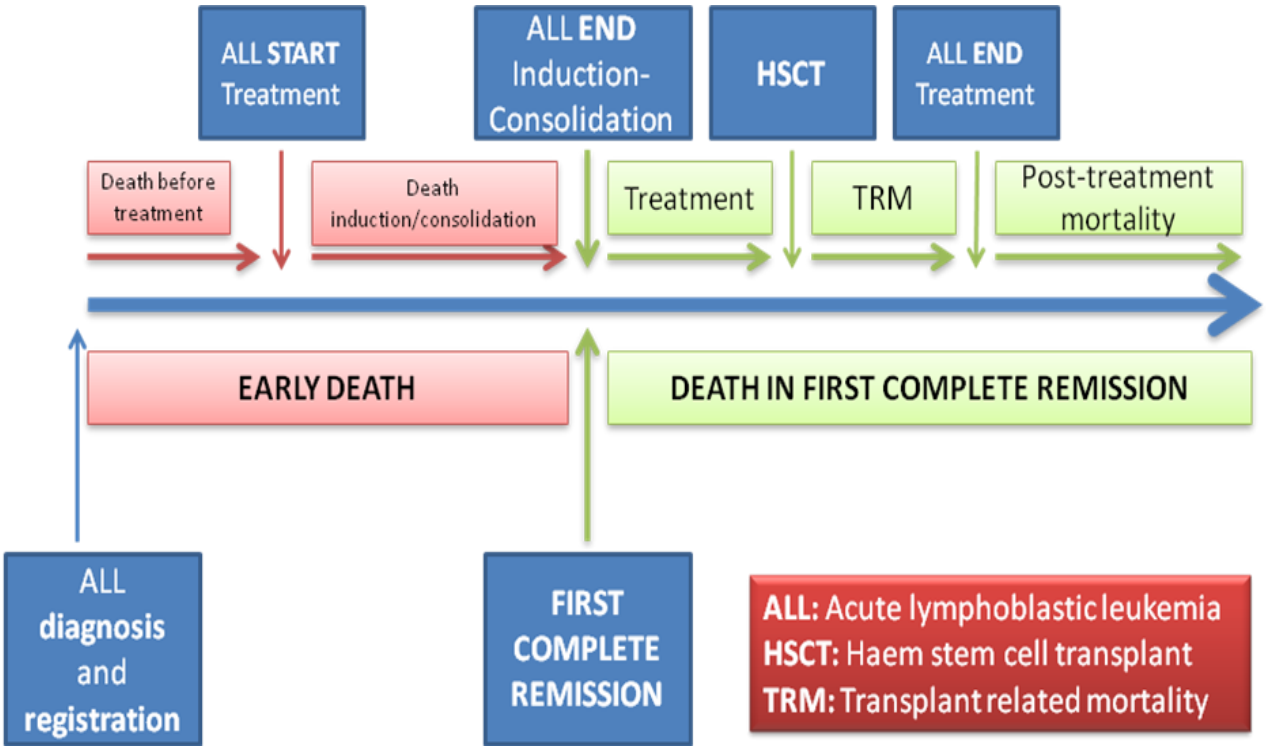


Figure 3. Study group definitions flowchart

The causes of death were grouped as:

- **Tumor burden related deaths:** Included tumor lysis syndrome (TLS) and leukostasis with compromised organ function due to infiltrating blast cells (e.g. mediastinal mass).
- **Infections:** Within this group, five major sub-groups were defined

    - Bacterial / Viral / Fungal / *Pneumocystis jirovecii* / Others or unknown.
- **Bleeding or thrombosis related deaths:** All hemorrhages, including those associated to leukostasis (e.g. intracerebral infiltration of leukemic blasts and intracerebral bleeding) were included in this group. Bleeding and thrombosis episodes were described as separate events for further clarification despite begin considered within the same group for the purposes of the analysis.
- **Organ toxicity not induced by infection** (e.g. anthracycline induced cardiotoxicity, asparaginase induced pancreatitis).
- **Transplant related mortality (TRM):** TRM has been defined as any cause of death in patients who underwent HSCT in first remission that could be directly attributed to the procedure itself. The ultimate cause of death associated to transplant (e.g. graft versus host disease, hemorrhages) was described. Infections leading to death after transplant were included within this group. EBV driven post-transplant lymphoproliferative disorder (PTLD) has been included within this group.
- **Secondary malignant neoplasms (SMN):** SMNs were defined as any malignancies having occurred after initial ALL and distinct from it whatever the remission status of the patient was. SMNs were not prospectively recorded in trials 58741, 58831/2 and 58881. SMNs were categorized following standard international recognized classifications for oncological diseases in children[2].
- **ALL progression:** In patients who died before remission and for which the investigators could not address any of the other causes described here.
- **Others or uncertain causes:**
    - Others in the group of Early Death included non-infectious neurological insults (stroke, cerebral edema, brain hernia, and encephalopathy), adult respiratory distress syndrome (ARDS), veno-occlusive disease (VOD), iatrogenic and unknown causes.
    - Others in the group of Death in Complete Remission included ARDS, aspiration, cardiac arrhythmias, sudden death, traffic accidents, suicide, iatrogenic and unknown causes.

For the purposes of this study, 'histiocytic disorders'[77] including hemophagocytic lymphohistiocytosis (HLH), macrophagic activation syndrome (MAS) and other systemic histiocytosis were not considered within the group of secondary malignancies and analyzed aside. If they were the cause of death, they were included in this group of others.

1.4 National Cancer Institute (NCI) group definitions

Patients were initially classified according to the specific risk criteria defined in each protocol. In order to homogenize groups under the same risk criteria and to compare with the results reported by other groups, patients were also classified according to the NCI risk criteria[78] for children with ALL in:

- **Standard group:** Those aged between ≥1 and <10 years or with an initial WBC $<50x10^9$.
- **High-risk group:** Those aged <1 or ≥10 years or with an initial WBC $\geq 50x10^9$.

1.5 Age/Time definitions

Age at the time of recruitment was calculated based on a 365.25 day-year period as follows:

- Start date was date of birth.
- End date was the minimum value among date of registration, date of diagnostic bone marrow (BM) or peripheral blood (PB) and date of start any antileukemic therapy (prephase or induction).
- Conversion between days and years was made by dividing the number of days (difference between start and end dates) by a factor of 365.25 according to the EORTC statistics guidelines.
- For protocol 58741, age was calculated using date of birth as the start date, and date of starting consolidation as the end date because only patients who achieved a CR after induction (and therefore able to proceed to the consolidation) were to be included in the protocol.

Any other calculations involving dates were made based on these same premises.

1.6 Disease definitions

- **Acute lymphoblastic leukemia (ALL):** Any disease associated with the presence of ≥ 25% of lymphoblasts in at least one bone marrow aspirate.
- **Non-Hodgkin Lymphoma (NHL):** Any neoplastic proliferation within the lymphoid cell lineage with less than 25% lymphoblasts in the bone marrow.

  The diagnosis for ALL/NHL was based on bone marrow aspirates by means of cytology, cytochemistry.
- **Central nervous system (CNS) involvement:** The definitions used here are based on international accepted definitions. Apart from the CNS status there are also CNS involvement definitions (taking into account other involvements). Recommendations were done in order to assure that any lumbar punction was done under a stable haemostatic condition and an experienced physician did that.
    - **Initial CNS status (Cerebrospinal fluid findings(CSF) at Day 1)**
        - For **non-traumatic punctures**, CNS status is defined as follows:
            - **CNS 1:** nontraumatic puncture, ≤ 5 WBC/µl CSF without leukemic cells on cytospin.
            - **CNS 2 (Surreptitious):** nontraumatic puncture, ≤ 5 WBC/µl CSF with identifiable leukemic cells on cytospin.
            - **CNS 3:** nontraumatic puncture, > 5 WBC/µl CSF with identifiable leukemic cells on cytospin.
        - For **traumatic lumbar punctures**, CNS status is defined as follows: A traumatic lumbar puncture (TLP) is defined as 10 or more erythrocytes/µl CSF or as CSF macroscopically contaminated with blood.
            - **TLP +:** traumatic lumbar puncture with leukemic cells on cytospin.
            - **TLP -:** traumatic lumbar puncture without leukemic cells on cytospin

- **'Late' CNS status (beyond the 1st IT injection)**
  - For patients with initial CNS 2 at Day 1:
    - **"Late CNS 1":** Patients with initial CNS 2 at day 1 becoming CNS 1 or TLP - at the second lumbar puncture (mostly Day 4 of prephase)
    - **"Late CNS 3":** Patients with initial CNS 2 at day 1 remaining CNS 2 or becoming CNS 3 or TLP + at the second lumbar puncture at the second lumbar puncture (mostly Day 4 of prephase)
  - Patients with initial TLP + at Day 1:
    - **'TLP+ Late CNS 1':** Patients with initial TLP+ becoming CNS 1 or TLP- at the second lumbar punction (mostly Day 4 of prephase)
    - **'TLP+ Late CNS 3':** Patients with initial TLP+ remaining TLP+ or becoming CNS2 or CNS3 at the second lumbar puncture (mostly Day 4 of prephase)
- **Definitions of CNS disease:**
  - **CNS involvement:**
    - CNS 3 status (All CNS status including 'Late' CNS 3)
    - Intracerebral or meningeal leukemic (proven or probably) mass seen on the MRI or CT scans
    - Cranial nerve palsy (irrespective of CSF or imaging findings)
  - **Surreptitious (CNS 2):** As defined above
  - **Dubious:** In case of hemorrhagic spinal tap, CNS involvement is called dubious when the following three criteria are met:
    - > 5 leukocytes/mm^3 and
    - > 50% leukemic cells on cytospin
    - Ratio erythrocytes / leukocytes >10.

- **ALL morphology classification:** Was made according to the French-American-British morphology classification (FAB)[5]

- **NHL morphology classification:** Was made according to the Revised European-American classification of lymphoid neoplasms (REAL)[79].

1.7 Response criteria

- **Complete remission (CR):**
    - Disappearance of all symptoms and of all physical and radiological signs related to the leukemia or the NHL.
    - < 5% blasts in the bone marrow with either normal cellularity or moderately reduced cellularity with signs of recovering myelopoiesis and thrombocytopoiesis in the peripheral blood.
    - CSF: No blasts.
- **Good partial response (GPR):**
    - Disappearance of all clinical, imaging and cytological signs of lymphoma or of leukemia except in one tumor site with persistence of a residual mass, the main diameter of which must not exceed 30% of the initial diameter.
- **Partial response (PR):**
    - In ALL: Any response less than CR, with 5.1%-25% blasts in the bone marrow at completion of induction.
    - In T-lineage ALL: even if the number of blasts in the bone marrow is < 5%, there is a partial response in case of a residual mass, the main diameter of which, exceeds 30% of the initial diameter.
    - In NHL: any response less than GPR at completion of induction.
- **Resistance:**
    - In ALL: Blasts in the bone marrow > 25% at completion of induction or PR at completion of induction and failure to achieve a CR at completion of consolidation.
    - In NHL: No response (no CR nor GPR) after induction or progression from GPR after consolidation or failure to achieve CR/GPR after consolidation.
- **Bone marrow relapse:**
    - Reappearance of more than 20% leukemic blasts cells in the BM.

- In case of leukemic blasts between 5% and 20% a control with more than 20% was necessary to ascertain the diagnosis of relapse.

- **Extra-medullary relapse:**
  - Reappearance of leukemic blasts or lymphoma cells in any extra-medullary tissue.

1.8 Treatment guidelines and follow-up

For all trials a detailed written protocol was provided to all investigators and participating centers.

Standard supportive care guidelines including management of hyperleukocytosis or bulky disease were provided in each protocol. Institutional guidelines for supportive care where applied individually in each participant center at physicians' discretion.

Recommendations for specific parts of trial such as HSCT were provided including selection criteria for transplant, selection of donors, conditioning chemotherapy and prevention of GVHD. Only reference centers for autologous or allogeneic transplantation were allowed to perform such procedure.

Specific *Pneumocystis jirovecii* prophylaxis was recommended in all trials except for the 58741.

Strict rules were implemented at the end of each phase of the treatment to proceed further.

In order to avoid the inadvertent administration of IT injection of Vincristine, one of the most common errors reported by other groups[80], the day of the administration of any IT chemotherapy did not coincide with the day of administration of IV Vincristine since protocol 58951.

Follow up monitoring was performed by sending out questionnaires from participating centers on a yearly or bi-yearly basis. In case any other event occurred meanwhile, the corresponding questionnaire was filled up and sent.

1.9 Ethical considerations and patient informed consent

The corresponding Institutional Review Board and Ethics Committee at each site approved the different clinical trials described in this study.

All patients, parents or legal guardians were informed of the aims of the different EORTC ALL-CLG trials, the possible adverse events (AE), the procedures and possible hazards to which he/she was exposed, and the mechanism of treatment allocation.

They were informed as to the strict confidentiality of their patient data, but that their medical records could be reviewed for trial purposes by authorized individuals other than their treating physician. Data was properly anonymized.

Written informed consent was obtained from parents or legal guardians of all patients included in the EORTC ALL-CLG trials described in this study before they were registered at the EORTC Data Center.

That was done in accordance with the national and the local regulatory requirements in practice at each time period.

1.10 Statistical analysis

Definitions:

- Follow up was calculated using the date of diagnosis as the starting date and the date of last follow-up (date of death or last day known to be alive) as the ending date. If the date of diagnosis was not available, the minimum date among date of registry or date of start treatment was used instead.
- Time to death was calculated using the date of diagnosis as the starting date and date of death as the end date. If the date of diagnosis was not available, the minimum date among date of registry or date of start treatment was used instead.
- Time to relapse was calculated using the date when a first CR was achieved after induction or consolidation as the starting date and the date of first relapse as the end date.
- Time to death in first CR was calculated using the date when the first CR was achieved after induction or consolidation as the starting date and the date of death in first CR as the end date.
- Relapse free survival (RFS) was calculated from the date of CR until the date of first relapse or in case no event occurred, until the day of last follow-up; patients who did not reach CR either after the induction IA or the consolidation IB or IB' were considered as events at time 0.

- Event free survival (EFS) was calculated from the date of CR until the date of first relapse or until death in CR, or in case no event occurred, until the day of last follow-up; patients who did not reach CR either after the induction IA or the consolidation IB or IB' were considered as events at time 0.
- Overall survival (OS) was defined as the time from diagnosis to death of any cause or last follow-up if they remained alive.

Statistical procedures:

- Probabilities of survival (pEFS and pOS) were estimated using the Kaplan-Meier method.
- Patients' baseline characteristics were summarized as number (percentage) of patients for categorical variables and median (range) for continuous variables. Differences in clinical variables were studied by $\chi 2$ test or Fisher exact test for categorical data. Mean quantitative variable differences were compared using Student's t test. The assumption of normality was tested by the Shapiro-Wilk test. Levene's test was calculated to compare the equality of variances. If the data failed the normality test, thus the Wilcoxon signed rank sum test was performed. All *p* values were two-sided. *P* values were considered statistically significant when they were <0.05.
- For the analysis of risk factors for death before remission and death in complete remission a multivariate analysis via logistic regression analysis was performed.
- Calculations of risk factors in first remission was done by excluding patients who did not reach complete remission, as they were not at risk of dying in complete remission and censoring patients who relapsed at the time of these events.
- In order to identify the risk factors for early death, the following baseline characteristics were compared between those who died before remission and those who did not, excluding all patients from trial 58741, because they were included in the trial once in remission, and the 58951 because data for this analysis was not available. Variables included in this analysis were: age at diagnosis, gender, WBC at diagnosis, immunophenotype, protocol, NCI risk and CNS involvement
- In order to identify the risk factors for dying in first complete remission, the following baseline characteristics were compared between those who died in

remission and those who did not, excluding all patients who did not achieve a first CR after induction: protocol, age at diagnosis, gender, WBC at diagnosis, immunophenotype, NCI risk, CNS involvement and having been transplanted in first CR. Data from the 58951 trial was not used for this analysis because it was not available. Trials 58831/58832 and the 58881 were grouped for analysis of risks in remission because the 58741 notably differed from the two others both in the period in which was performed and in the background treatment.

- Statistical analyses were performed using SPPS 21® software.

1.11 Literature search strategy and bibliography

Literature review was performed using 'leukemia', 'treatment', 'complications' and 'death' as MeSH search terms in PubMed. Search was limited using the advanced search function for articles published in 'all child' (0-18 years) and 'young adult' (19-24) and whose language was English, French, German or Spanish. No other restrictions were used. To identify additional publications, search was performed in EMBASE and The Cochrane Library, references from articles, review articles, reference pediatric oncology textbooks and communications to congresses or non-published data.

2. PROTOCOLS

2.1 Protocol 58741

Description

This study was undertaken in 1971. Based on previous evidence, the trial aimed to compare in a first step two different consolidation treatments for ALL patients who had already achieved a complete remission at the end of the induction therapy. In a second step, the trial compared two different approaches at the time of maintenance, chemotherapy or immunotherapy based.

Objectives of the trial

- To compare the effectiveness of two consolidations treatments in CR after induction of remission with vincristine (VCR) - prednisone (plus daunorubicin if necessary).

- To assess the effectiveness of immunotherapy in patients in complete remission after induction and consolidation treatment of ALL.

Eligibility criteria

Inclusion criteria:

- Patients aged between one and fifty years.
- Newly diagnosis of ALL induced in CR according to the protocol.

Exclusion criteria:

- Patients over 50 years of age.
- Patients whose induction treatment had been initiated elsewhere for more than 2 weeks or had received cytostatic drugs other than 6-Mercaptopurine (6-MP), corticosteroids, vincristine or daunorubicin.
- Patients with leukemic transformation of a lymphoma showing initially no blood or marrow transformation.
- Those who did not achieve a CR at the end of induction.

Randomization

- All randomizations took place at the EORTC data center in Brussels. In this particular trial randomizations were obtained by telephone or telex.
- At the time of randomization for consolidation, patients younger or older than 21 years were randomized separately in the different participating centers.
- At the time of randomization for maintenance treatment, patients assigned for consolidations P or M were randomized separately.

The patients eligible for the present study were randomly assigned to one of the two consolidations regimens, Polychemotherapy (P) or Methotrexate based (M), and 1 year later to maintenance by chemotherapy (C) or by immunotherapy (I).

Treatments

- Induction to remission treatment:

Induction therapy consisted of daily oral prednisolone (40mg/m^2) and weekly intravenous injections of VCR (2mg/m^2) for 4 weeks. If after 3 VCR injections, the bone marrow still contained 6%-15% blasts or 16%-25% blasts, the induction was prolonged to, respectively, 5 or 6 weeks. If after 3 injections of VCR, the marrow still contained >25% blasts, the induction was

prolonged to 6 weeks, and 2 weekly injections of daunorubicin 60mg/m^2 were added. If CR was not achieved within 8 weeks, the patient did not enter the trial. CR was defined as the absence of blasts in the blood, the absence of marrow aplasia, the presence of less than 5% blasts in the bone marrow, and the absence of leukemic infiltration on examination.

No prophylactic treatment of *Pneumocystis carinii* was given.

The reinduction therapy given to the relapsing patient was not standardized, since it was anticipated, and this was indeed the case, that relapse would occur during the administration of a variety of drugs.

- Consolidation treatment:

Here the term 'consolidation' will be used for a one-year therapy phase, despite in general this term has been used for a 1-12 week period of intensive treatment after remission induction.

Consolidation P (P for polychemotherapy) consisted of an 8 successive phases of chemotherapy given within a period of 12 or 13 months maximum. The first 4 phases given in 6 months were repeated during the next 6 months. Phases 1 and 5 (4 weeks) consisted of L-Asparaginase 150.000 IU/m^2 given IV once a week and 6-MP, 70 mg/m^2/day orally in 2 or 3 doses. Central nervous system (CNS) prophylaxis was administered during phase 1. Phases 2 and 6 (8 weeks) consisted of prednisone (PDN) 40mg/m^2/day orally in 3 or 4 doses, and 6-MP 70mg/m^2/day orally in 2 or 3 doses. Phases 3 and 7 (8 weeks) consisted of bis-chlorethyl-nitrosureum (BCNU), 50mg/m^2 once a month IV for phase 3 and 100mg/m^2 once a month IV for phase 7, and cyclophosphamide (CPM), 70 mg/m^2/day orally for 8 weeks. Phases 4 and 8 (6 weeks) consisted of methotrexate (MTX), 15-20 mg/m IM or orally, twice a week, and VCR 1mg/m IV once a week.

Consolidation M (M because methotrexate was the major agent) consisted in 7 successive phases of chemotherapy given within a period of 12 or maximum 13 months. The 7 phases were identical and consisted of 30 days intermittent MTX followed by 21 days of reinforcement with PDN and VCR. MTX was given at 15mg/m^2 IV bolus injection during 3, 4 or 5 consecutive days, depending on the tolerance. Three sequences of 3, 4 or 5 days separated by at least 5 days of therapy had to be given over a period of 1 month. Reinduction was repeated after every 30 days of intermittent MTX: VCR 2mg/m^2 IV at days 1, 8 and 15, and prednisone, 40 mg/m^2/day

orally at days 1-15 and then decreased progressively on days 16 and 17. MTX was restarted at day 21 of reinduction.

The doses were reduced in case of toxicity. The L-Asparaginase injections were spaced when fibrinogen fell below 50mg/dl and were reduced to 40.000 IU/m^2/week or discontinued according to severity of transaminase, amylase, glucose or CNS abnormalities. For hematological toxicity, when WBC was >3000/µl and platelets >100.000/µl all drugs could be given at 100% of the dose. Below 1.000 WBC/µl and 50.000 platelets/µl, all therapy was stopped. Between these thresholds, adjustments of the doses were recommended: with WBC between 2.000 and 3.000/l and/or platelets between 50.000 and 100.000/l, the dose of MTX, 6-MP, CPM and BCNU was reduced by 50%. Between 1.000 and 2.000 WBC/µl MTX, 6-MP and CPM were stopped, with Asparaginase, VCR and BCNU (first cycle) being reduced to 50% of the original dose. When, for consolidation M, WBC was 2.000-3.000/µl, only 3 days of MTX were give, but when WBC was below 2.000/µl, MTX was delayed until WBC reached 2.000/µl.

- Prophylaxis of CNS relapses:

Prophylactic therapy of CNS leukemia was given to all patients immediately after CR was achieved, during the begging of consolidation P or M. Before July 1973, patients received 12 monthly intrathecal injections of MTX (5mg/m^2) and cytarabine (10mg/m^2) and craniospinal irradiation, 1.500 rads to the skull and 1.000 rads to the spine. After that date, based on published promising results by Aur et al[81], the regimen was changed to 2.400 rads cranial irradiation combined with 5 intrathecal injections of MTX (12mg/m^2) given over a 16 day period.

- Maintenance treatment:

The patients who were still in continuous CR after 1 year of consolidation were further randomized, separately for each consolidation arm, to receive either maintenance chemotherapy or immunotherapy for a period of 4 year.

Maintenance chemotherapy consisted of daily 6-MP (90mg/m^2) and weekly oral or intramuscular MTX (15/m^2). These drugs were stopped for 21 days when reinforcement was given: prednisone (40mg/m^2 orally on days 1-5) and VCR (1.5 mg/m^2 IV on days 1, 8 and 15). These reinforcements were given every 3 months the first year, every 4 months the second year, and every 6 months thereafter.

Patients on immunotherapy received a combination of a) 2ml of fresh fluid Bacillus Calmette-Guerin (BCG) (From the Institute Pasteur, Brussels) spread over a 20 x 5 cm scarification on one leg. The preparation contained 8 x 10^7 viable units/ml and was administered twice weekly for 6 months and once a week thereafter; b) allogeneic non irradiated leukemic blasts, 4 x 10^7 blasts injected intradermally once a week for the first 3 months and then once a month. The blast cells were obtained from one or several leukemic donors before treatment and cryopreserved at -196ºC in the presence of 10% dimethylsulfoxide (DMSO). DMSO was not washed out prior to the administration to the patient.

Both types of maintenance treatment were stopped after 4 years, i.e. 5 years from the beginning of consolidation.

A representative schema of the trial is shown in Figure 4.

Treatment details are depicted in table 3.

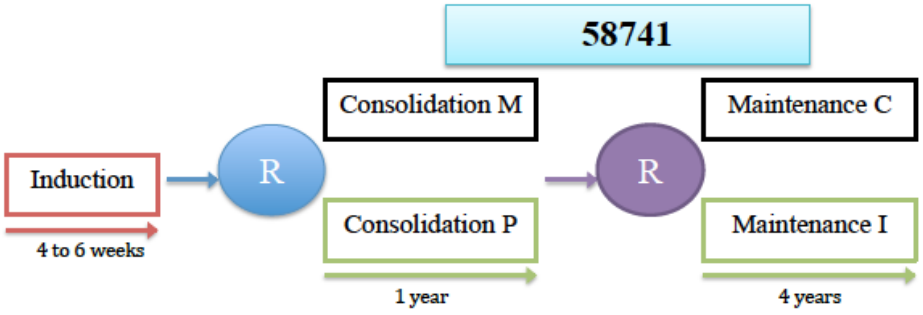


Figure 4. Trial 58741 schema

Table 3 - EORTC-CLG 58741: Treatment protocol for all patients		
Treatment phase/drug	**Dose**	**Days Given**
Induction		
- Prednisone[a]	140mg/m/day^2	1-28
- Vincristine	2mg/m^2	8,15,22,29
- Daunorubicin	60mg/m^2	36,43
P Consolidation		
Phase I/V (Weeks 1 and 5)[b]		
- L-Asparaginase (IV)	150.000 IU/m^2	8,15,22,29
- 6-Mercaptopurine	70mg/m^2	1-28
Phase II/VI (Weeks 2 and 7)		
- Prednisone	40mg/m^2	1-42
- 6-Mercaptopurine	70mg/m^2	1-42
Phase III/VII (Weeks 3 and 7)		
- BCNU (IV)	50mg/m^2	
- Cyclophosphamide (PO)	70mg/m^2	1-42
Phase IV/VIIII (Weeks 4 and 8)		
- Methotrexate (PO)	15-20mg/m^2	1,8,15,22,29,36,43,50,57,64,71,78
- Vincristine	1mg/m^2	8,15,22,29,36,43
M consolidation		
- Methotrexate (Weeks 1-4)[c]	15mg/m^2	1, 11, 21
- Vincristine (Weeks 5-9)	2mg/m^2	1,8,15
- Prednisone (Weeks 5-9)	40mg/m^2	1-15
C Maintenance		
- 6-Mercaptopurine[d]	90mg/m^2	Once a day
- Methotrexate (PO)	15mg/m^2	Once a week
- Vincristine	1.5mg/m^2	1, 8, 15
- Prednisone	40mg/m^2	1-15
I Maintenance		
- Bacillus Calmette-Guerin	2ml(8x10^{7}IU/ml)	Twice weekly for first 6 months and once a week afterwards
- Allogenic blasts (ID)	4 x 10^7 blasts	Once a week for first 3 months and once a month afterwards

IV: s **PO:** Per os; **ID:** Intradermal

[a] Prednisone (PDN) and Vincristine (VCR) were to be prolonged up to 8 weeks depending on the response observed in the BM at 4 and 6 weeks respectively. Daunorubicin was added at week 4 of treatment in case >25% blasts were observed in the BM.

[b] CNS prophylaxis was administered during phase I of P consolidation and during first course of M consolidation and consisted of 2.400 rads cranial irradiation combined with 5 intrathecal injections of Methotrexate (MTX) (12mg/m^2) given over a 16 day period.

[c] Three courses of MTX were given over a period of 30 days followed by a 21 days reinduction period of VCR and PDN. On day 21 a second course of MTX was started followed by its corresponding reinduction. This sequence was repeated 7 times over a 12 to 13 months period.

[d] Maintenance chemotherapy consisted on daily 6-Mercaptopurine (6-MP) and weekly MTX. These drugs were stopped when reinforcement with VCR and PDN were given. Reinforcement was administered every 3 months the first year, every 4 months the second year, and every 6 months thereafter.

2.2 Protocols 58831/58832

Description

From 1983 to 1989, all children with ALL and NHL under the age of 18 years of age were included in one of the two studies: CLG-EORTC 58831 for standard risk patients and 58832 for medium and high-risk patients. These trials were based on BFM-like protocols, in which the treatment stratifications relied on three initial factors: the number of circulating blasts, the size of the liver and of the spleen below the costal margin. Risk factor (RF) calculation was carried out according to Langerman[82] (RF = 0.2 x $\log^{10}$ (blasts/mm^3 + 1) + 0.06 x cm hepatomegaly + 0.04 x cm splenomegaly). The protocol 58831 for standard risk (SR) ALL was designed for patients with a RF below 1.2, whereas medium (MR) (RF between 1.2–1.69) and high-risk (HR) ALL patients (RF >1.7) were treated according to the more intensive protocol 58832.

Objectives of the trial 58831

- To investigate if the BMF regimen remains equally efficient after deletion of cyclophosphamide from the initial intensive phase of treatment.
- To evaluate the possible improvements in short term and long term toxicity after deletion of cyclophosphamide.

Eligibility criteria 58831

Inclusion criteria:

- All patients less than 18 years of age with newly diagnosed (non-B) ALL with standard risk (SR) characteristics, i.e. with a risk factor (RF) < 1.2, who achieve a CR at completion of induction therapy (IA).

Exclusion criteria:

- Patients with B-cell ALL, as identified by the presence of surface immunoglobulins on the leukemic blasts.
- Previously treated patients, unless the treatment was limited to corticoids and/or vincristine, was shorter than duration of 8 days, and if the diagnosis of SR ALL can could be established with the available clinical and cytological data.
- Patients with NHL, which subsequently developed into leukemia.
- Patients with NHL and bone marrow invasion.

- Patients with severe encephalopathy, severe heart disease or trisomy 21.
- Patients with initial involvement of the CNS.
- Patients who did not achieve CR within 35 days of induction therapy. These patients were eligible for protocol 58883.

Objectives of the trial 58832

- To investigate whether subclinical leukemic involvement of the CNS could be equally eradicated by high dose methotrexate alone as by high dose methotrexate followed by radiotherapy to the cerebral meninges in ALL children with medium and high-risk characteristics. To answer this question, the therapeutic protocol provided high doses methotrexate (500mg/m^2) given four times concomitantly with intrathecal methotrexate to all patients. Subsequently, the patients were randomized to receive or not to receive radiotherapy to the brain.

Eligibility criteria 58832

Inclusion criteria:

- All patients less than 18 years of age with newly diagnosed (non B) ALL with medium and high-risk characteristics who achieved CR at completion of protocol I phase B and remained in CR at completion of reinduction protocol II.
- Patients with initial SR characteristics who did not achieve CR at completion of protocol I, phase A but did achieve CR at completion of protocol I, phase B and remained in CR up to the time of randomization (end of protocol II).

Exclusion criteria:

- Patients with B-cell ALL, as identified by the presence of surface immunoglobulins on the leukemic blasts.
- Previously treated patients, unless the treatment was limited to corticoids and/or vincristine, was shorter than duration of 8 days, and if the diagnosis of MR and HR ALL can could be established with the available clinical and cytological data.
- Patients with NHL, which subsequently develops into leukemia.
- Patients with NHL and bone marrow invasion.
- Patients with severe encephalopathy, severe heart disease or trisomy 21.
- Patients with initial involvement of the CNS.

- Patients who did not achieve CR within completion of protocol I, phase B.

Randomization

All randomizations took place at the EORTC data center in Brussels. In this particular trial randomizations were obtained by telephone or telex.

Patients in the 58831 trial were randomized at the time of phase I according to center.

Patients in the 58832 trial were randomized during protocol II phase b according to center and to category of risk (MR and HR respectively).

Treatments

Treatment details for both protocols are here described:

Induction therapy (protocol IA) consisted of 4-weekly vincristine and daunorubicin, daily prednisolone for 4 weeks and daily i.v. infusion of asparaginase for 21 days. This was followed by a 4-week early consolidation (protocol IB) that included daily 6-mercaptopurine for 28 days and Cytarabine for 4 consecutive days each week. Patients with standard risk ALL were randomized to receive or not cyclophosphamide (1 g/m2 on days 1 and 29 of protocol IB). All medium and high-risk children received this alkylating agent. After completion of IB, the interval therapy was applied consisting of an 8-week course of 6-mercaptopurine p.o. and four administrations of high-dose methotrexate injections, 500 mg/m2 for standard risk patients and 2500 mg/m2 for medium and high-risk patients. Finally, standard risk patients (protocol 58831) received a 1-month late intensification treatment that included dexamethasone for 14 days, two doses of doxorubicin and vincristine and four doses of asparaginase followed by cytarabine (two cycles as in consolidation) and 6-thioguanine. For medium and high-risk patients (trial 58832) this late intensification lasted 6 instead of four weeks (protocol IIA and IIB) and was the same as trial 58881. For standard risk patients, a total of six IT methotrexate injections were scheduled during these phases of treatment and, for median and high-risk patients, seven IT injections. Maintenance therapy was pursued with 6-mercaptopurine daily and oral methotrexate weekly, without IT treatment, for duration of 2 years from the start of induction therapy. Medium and high-risk patients were randomized into two groups, for receiving or not prophylactic cranial radiation after the completion of protocol II and before the start of maintenance therapy. Children above 2 years of age received 24 Gy, the dose was reduced for younger patients (20 Gy

between 1 and 2 years of age). Patients with CNS manifestations at diagnosis were not eligible in trial 58831/58832.

Evaluation of response was made at the end of Induction (IA) and end of Consolidation (Protocol IB). Patients who did not achieve a CR at the end of IA were meant to continue into IB. Patients who either achieved a CR at the end of IA or IB were considered responders to therapy and allocated to the corresponding trial as specified in the inclusion/exclusion criteria.

Patients with high-risk features could be considered for transplantation in first remission. The decision to proceed to transplantation was based on the investigator criteria at each center. For the purposes of this study, only patients having achieved a first complete remission after protocol Ia or Ib and having been decided to receive a transplant were included in the analysis of toxic deaths.

Representative schemas of the trial are shown in Figures 5 and 6.

Treatment details are depicted in tables 4 and 5.

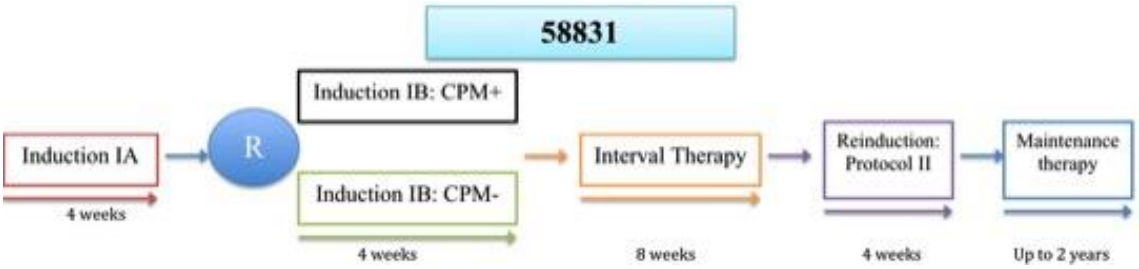


Figure 5. Trial 58831 schema

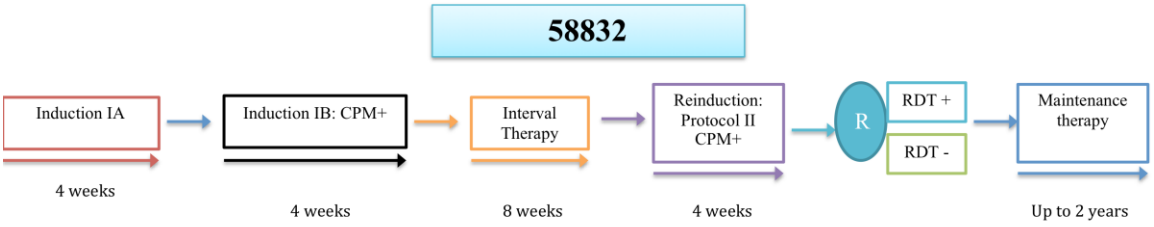


Figure 6. Trial 58832 schema

Table 4 - EORTC-CLG 58831: Treatment protocol for standard risk patients (SR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	1-28
- Vincristine	1.5mg/m^2	1,8,15,22
- Daunorubicin	30mg/m^2	1,8,15,22
- Asparaginase (IV)	5000 IU/m^2	1-21
- Methotrexate (IT)	12mg (age-dependent)	1
Protocol IB		
- Cyclophosphamide according to randomization	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Methotrexate (IT)	12mg (age-dependent)	39
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate	500mg/m^2	8,22,36,50
- Methotrexate (IT)	12mg (age-dependent)	8,22,36,50
Reinduction: Protocol II		
- Dexamethasone	10mg/m^2	1-14
- Vincristine	1.5mg/m^2	1,8
- Doxorubicin	30mg/m^2	1,8
- Asparaginase (IV)	10000 IU/m^2	1,4,8,11
- Cytarabine	75mg/m^2	17-20, 24-27
- 6-Tioguanine	60mg/m^2	15-28
Maintenance		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly

IT: Intrathecal**; IV:** Intravenous; **PO:** Per os

Table 5 - EORTC-CLG 58832: Treatment protocol for medium (MR) and high-risk (HR) patients		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	1-28
- Vincristine	1.5mg/m^2	1,8,15,22
- Daunorubicin	30mg/m^2	1,8,15,22
- Asparaginase (IV)	5000 IU/m^2	1-21
- Methotrexate (IT)	12mg (age-dependent)	1
Protocol IB		
- Cyclophosphamide	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Methotrexate (IT)	12mg (age-dependent)	39
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate	2.5g/m^2	8,22,36,50
- Methotrexate (IT)	12mg (age-dependent)	8,22,36,50
Reinduction:		
Protocol IIA		
- Dexamethasone	10mg/m^2	1-28
- Vincristine	1.5mg/m^2	1,8, 15, 22
- Doxorubicin	30mg/m^2	1,8, 15, 22
- Asparaginase (IV)	10000 IU/m^2	1,4,8,11
Protocol IIB		
- Cyclophosphamide	1g/m^2	29
- Cytarabine	75mg/m^2	31-34, 38-41
- 6-Tioguanine	60mg/m^2	29-42
Maintenance[a]		
- 6-Mercaptopurine	50mg/m^2	Daily
- Methotrexate	20mg/m^2	Weekly

IT: Intrathecal**; IV:** Intravenous; **PO:** Per os

[a] Medium and high-risk patients were randomized into two groups, for receiving or not prophylactic cranial radiation after the completion of protocol II and before the start of maintenance therapy.

2.3 Protocol 58881

Description

From 1989 to 1998 patients with ALL were included in the 58881 protocol. Based on previous evidence obtained from preceding EORTC trials and other groups, the CLG decided to run this randomized trial to test the efficacy of two different asparaginase formulation in front line therapy, the value of the addition of high doses cytarabine to the interval therapy and the value of alternation intravenous and oral administration of 6-mercaptopurine when compared to exclusive administration alone during maintenance.

Objectives

- To test the relative efficacy and toxicity of two different Asparaginase formulations (*Erwinia* Vs. *E. Coli*) in front line therapy in children with ALL and NHL
- To test whether the addition of high-dose cytarabine to interval chemotherapy improved the disease-free survival and the central nervous system-free interval in patients with increased risk non B-cell ALL, and in patients with stage III or stage IV non-B NHL.
- To test whether alternation of intravenous and oral administration of 6-mercaptopurine as compared to exclusive oral administration of this drug, during maintenance, improved the disease-free survival and the central nervous system free interval, in all patients with non-B cell ALL and non-B cell NHL (excluding very high-risk patients).

Eligibility criteria

Inclusion criteria:

- All patients less than 18 years of age, with non B-cell ALL or with non B-cell NHL (any stage).

Exclusion criteria:

- Patients previously treated, unless the treatment consisted of corticoids only and did not exceed 7 days in duration, and clinical as well as hematologic data were available allowing for correct staging of the patients with B-cell ALL or B-cell NHL.

  B-cell ALL was defined by L3 morphology in the FAB classification and by the presence of immunoglobulins on the membrane or in the cytoplasm of at least 20% of

the leukemic blasts or by the presence of translocations specific for B-cell neoplasias, i.e. t (2;8), t (8;14) or t (8;22). All ALL with a less mature immunophenotype within the B-cell lineage, including those with cytoplasmic μ-chains and L1 or L2 morphology (i.e. pre-B-cell ALL), were eligible. The very rare cases of ALL with L1 or L2 morphology and immunoglobulins on the membrane were eligible for this study. In patients with NHL where the immunophenotype was not available, the diagnosis of B-cell NHL could be established on cytological and histological criteria, according to the Kiel classification[83].

Eligibility criteria for the randomized trial pertaining Asparaginase Therapy

Inclusion criteria:

- All patients less than 18 years of age, with non B-cell ALL or with non B-cell NHL (any stage).

Exclusion criteria:

- Patients previously treated, unless the treatment consisted of corticoids only and did not exceed 7 days in duration, and clinical as well as hematologic data were available allowing for correct staging of the patients with B-cell ALL or B-cell NHL.

Eligibility criteria for the randomized trial pertaining to Interval Therapy

Inclusion criteria:

- All patients with increased risk non-B ALL (non-T with an risk factor > 0.8 and all T ALL, excluding very high-risk patients) or with stage III or stage IV non-B NHL, who achieved CR by the end of protocol Ia (induction) and remained in CR after completion of protocol Ib (consolidation).

Exclusion criteria:

- Low risk non-B non-T ALL patients, i.e. with R.F. < 0.8.
- Stage I or stage II NHL.
- Patients not in first CR.
- Very high-risk patients.
- Patients with non-equivocal or overt initial involvement of the CNS.

Eligibility criteria for the randomized trial pertaining to Maintenance Therapy

Inclusion criteria:

- All patients, with non B-cell ALL of any R.F. (excluding very high-risk patients only) or with non B-cell NHL of any stage, who achieved CR by the end of protocol Ia (induction) and remained in first CR after completion of protocol II (reinduction).

Exclusion criteria:

- Patients not in first CR.
- Very high-risk patients.
- Patients with non-equivocal or overt initial involvement of the CNS.

Eligibility criteria in the pilot study for Very High-risk Patients

Inclusion criteria:

- All patients with non B-cell ALL or non B-cell NHL, with any one of the following very high-risk features:
    - Bad response to prednisolone (or prednisone) prephase therapy (i.e. with the persistence of more than 1,000/mm3 leukemic blasts in the peripheral blood after 7 days of prephase therapy).
    - Absence of complete remission after completion of induction (by the end of protocol Ia). This failure to achieve CR had to be established by a bone marrow examination at day 42, before the start of Ib.
    - Undifferentiated immunophenotype, i.e., negativity for B (including CD19, CD20, CD24) and T-cell markers and for CALLA (CD10) and for myeloid markers (acute undifferentiated leukemia or AUL).
    - Cytogenetic abnormalities: t (9;22) or t (4;11) translocation, or nearhaploidy.

Randomization

This process was done centrally (EORTC Data Center, Brussels) by telephone through the Euro-Code network.

For the first question, randomization was stratified according to center, disease (leukemia versus lymphoma), risk factor (< 0.8, 0.8-1.19, ≥1.2), and immunophenotype (B versus T lineage) for leukemia patients, and by Murphy stage (stage I-II versus III-IV) for lymphoma patients. Randomization was not stratified by the presence of t(9;22).

Subsequent randomizations were stratified according to treatment arm and initial risk factor or Murphy stage.

Treatments

Patients were initially randomized to *Erwinia*-asparaginase (Erwiniase, Ipsen, Maidenhead, United Kingdom) or *E coli*–asparaginase (Paronal, Medac, Hamburg, Germany for the Belgian centers, or Kidrolase, Bellon, Montrouge, France for the French and Portuguese centers, both produced by Kyowa Hakko, Tokyo, Japan). Physicians had to switch to the other asparaginase in case of allergy grade 1 or higher. In case of pancreatitis or thrombosis, asparaginase was eliminated from the treatment. This comparison was carried out between November 1990 and October 1993

All patients received the same induction regimen with the exception of Asparaginase during the study period. After that, and in view of the differences between the two Asparaginase groups, all patients received E. Coli unless otherwise contraindicated[84].

After completion of this treatment, patients who had more than 1000 blasts/mm^3 of blood at the end of the first week of prednisolone treatment, those who did not achieve complete remission, and those with a t(4;11) or t(9;22) translocation were considered to have a very high-risk of relapse and so were classed in a very high-risk (VHR) group. The remaining patients considered as standard risk of relapse (low and intermediate risk according to Langerman[82] (RF = 0.2 x $\log_{10}$ (blasts/mm^3 + 1) + 0.06 x cm hepatomegaly + 0.04 x cm splenomegaly) as in the 58831/2 trials) received the same treatment with a consolidation (protocol IB), an interval therapy and a protocol II.

The second question investigated the value of high doses of cytarabine (1 g/m^2, twice, at a 12 h interval) combined with high doses of methotrexate during the interval therapy. Only patients with no VHR feature, either with B cell phenotype and a RF 0.8, or those with T cell lineage were eligible for this second randomization, which was carried out between September 1989 and January 1996.

A total of 10 IT methotrexate injections were scheduled during the intensive phases of treatment, but no IT was planned during maintenance. Cranial radiation was systematically omitted even for patients with CNS manifestations at diagnosis (5 blasts/mm^3 in CSF: CNS-3 status). The latter patients received two additional IT injections during induction and two during consolidation, and one IT injection, combined with high-dose methotrexate, every 3 months, during maintenance. So, patients with CNS involvement at diagnosis received a total of 20 IT

methotrexate injections. The presence of CNS blast cells with fewer than five leukocytes per micro liter was considered as surreptitious (CNS-2 status). In case of traumatic spinal tap with blood admixture and the impossibility of deciding between blood contamination and probable involvement, it was considered as dubious. Further to the publication of the prognostic significance of low leukemic cells in the CNS[85] almost 40% of study population with surreptitious or dubious CNS involvement were treated the same as those with overt CNS involvement. VHR patients received an intensified treatment for 1 year consisting of an intensified consolidation IB, a 'VANDA' course, an interval therapy followed by two series of three chemotherapeutic courses (R1, R2, and R3) according to the BFM relapse protocol[86] which relied on rotational high dose pulses of chemotherapy. This VHR treatment contained alkylating agents (cyclophosphamide in consolidation and ifosfamide in R2 bloc). The cumulative anthracycline dose/m^2 was 220 mg daunorubicin and 16 mg mitoxantrone. The CNS directed chemotherapy included a total of 16 IT methotrexate injections, six of which were triple, with cytarabine and steroids, scheduled during this first year of intensified treatment and also 10 courses of high-dose methotrexate. For those with CNS leukemia at diagnosis, IT methotrexate was pursued during maintenance every 3 months (a total of 20 IT injections were given during the overall treatment). Cranial radiotherapy was not planned.

For VHR patients who have an HLA-identical donor, with a negative mixed lymphocyte culture, bone marrow transplantation in first complete remission was an alternative to the VHR protocol. VHR patients having received the Ia and the Ib protocol, whether in CR or in PR or with progressive disease, with a genotypically identical HLA donor were considered potential candidates for transplantation. The decision to proceed to transplantation was based on the investigator criteria at each center. For the purposes of this study, only VHR patients having achieved a first complete remission after protocol Ia or Ib and having been decided to receive a transplant were included in the analysis of toxic deaths.

The third randomization was made to test the advantage of adding monthly intravenous 6-mercaptopurine (1 g/m^2) to conventional maintenance therapy with daily oral 6-mercaptopurine (initial dose 50 mg/m^2) adjusted to maintain the leukocytes between 2000 and 3000/mm^3 and methotrexate 20 mg/m^2 once a week. All patients in complete remission after

protocol II or after the last R3 bloc for those qualified as VHR, were eligible for this third question, which included patients between November 1990 and November 1996.

For all patients, the total duration of treatment was two years.

Representative schemas of the trial are shown in Figures 7 and 8.

Treatment details are depicted in tables 6 and 7.

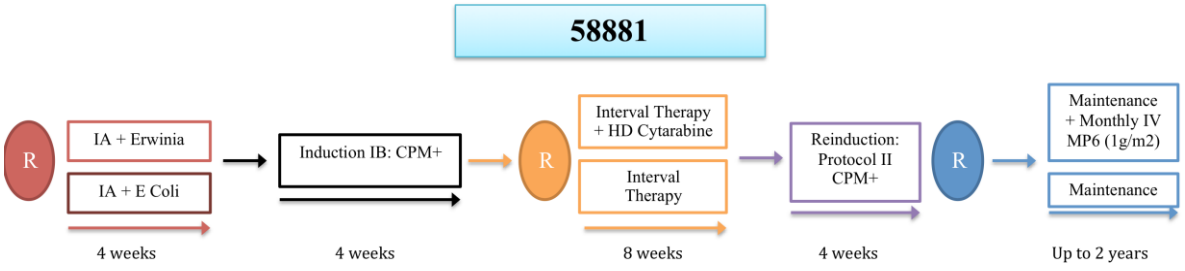


Figure 7. Trial 58881 schema

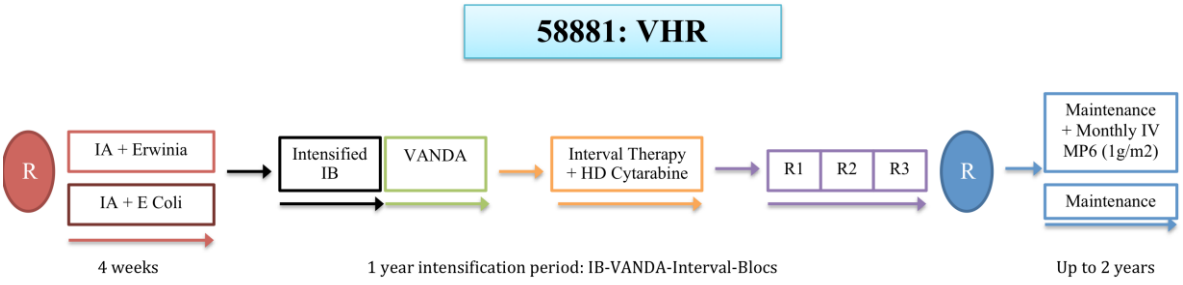


Figure 8. Trial 58881 schema VHR

Table 6 - EORTC-CLG 58881: Treatment protocol for standard risk patients (SR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	1-28
- Vincristine	1.5mg/m^2	8,15,22,29
- Daunorubicin	30mg/m^2	8,15,22,29
- Asparaginase (IV)[a]	10000IU/m^2	12,15,18,22,25,29,35,38
- Methotrexate (IT)	12mg (age dependent)	1,8,22,38,52
Protocol IB		
- Cyclophosphamide	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Methotrexate (IT)	12mg (age dependent)	39
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate (24 h infusion)	5g/m^2	8,22,36,50
- Methotrexate (IT)	12mg (age dependent)	9,23,37,51
- Cytarabine (according to randomization[b])	1g/m^2 (twice 12-h interval)	9,23,37,51
Reinduction: Protocol II		
- Dexamethasone	10mg/m^2	1-21
- Vincristine	1.5mg/m^2	8,15,22,29
- Doxorubicin	30mg/m^2	8,15,22,29
- Asparaginase (IV)	10000 IU/m^2	8,11,15,18
- Methotrexate (IT)	12mg (age dependent)	38
- Cyclophosphamide	1g/m^2	36
- Cytarabine	75mg/m^2	38-41, 45-48
- 6-Tioguanine	60mg/m^2	36-49
Maintenance[c]		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly

IT: Intrathecal**; IV:** Intravenous; **PO:** Per os

[a] Patients, regardless of their risk group, were randomly assigned to receive E. Coli asparaginase or Erwinia asparaginase at equal doses.

[b] Patients in CR, with an initial RF >0.8 or with a T-lineage ALL and without VHR features, were eligible for this randomization.

[c] Patients in CR after protocol II or after the last R3 bloc for those qualified as VHR were eligible for the third randomization to test the advantage of adding monthly intravenous 6-mercaptopurine (1 g/m^2) to conventional maintenance therapy.

Table 7 - EORTC-CLG 58881: Treatment protocol for very high-risk patients (VHR)		
Treatment phase/drug	**Dose**	**Days Given**
Protocol IB		
- Cyclophosphamide	1g/m^2	43,85
- Methotrexate	5g/m	43,57,71
- Cytarabine	1g/m^2	50,51,64,65,78,79
- Asparaginase	25000 IU/m^2	44,51,58,65,72,79
- 6-Mercaptopurine	25mg/m^2	43-84
- Methotrexate (IT)	12mg (age dependent)	44,58,72
VANDA		
- Dexamethasone	20mg/m^2	1-5
- Cytarabine	2g/m^2	1,2
- Mitoxantrone	8mg/m^2	3,4
- Etoposide	150mg/m^2	3,4,5
- Asparaginase	10000mg/m^2	7,9,11,13
- Methotrexate (IT)	12mg (age dependent)	5
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-42
- Methotrexate (24 h infusion)	5g/m^2	8,22,36
- Methotrexate (IT)	12mg (age dependent)	9,23,37
- Cytarabine	1g/m^2 (12-h interval)	9,23,37
Block R1		
- Dexamethasone	20mg/m^2	1-5
- 6-Mercaptopurine	100mg/m^2	1-5
- Vincristine	1.5mg/m^2	1,6
- Methotrexate	5mg/m^2	1
- Cytarabine	2mg/m^2	5
- Asparaginase	25000 IU/m^2	6
- MTX/Cytarabine/PDN (IT)	12mg/30mg/10mg	1
Block R2		
- Dexamethasone	20mg/m^2	1-5
- 6-Thioguanine	100mg/m^2	1-5
- Vindesine	3mg/m^2	1
- Methotrexate	5mg/m^2	1
- Ifosfamide	400mg/m^2	1-5
- Daunorubicin	50mg/m^2	5
- Asparaginase	25000mg/m^2	6
- MTX/Cytarabine/PDN (IT)	12mg/30mg/10mg	1
Block R3		
- Dexamethasone	20mg/m^2	1-5
- Cytarabine	2g/m^2	1,2
- Etoposide	150mg/m^2	3,4,5
- Asparaginase	25000IU/m^2	6
- MTX/Cytarabine/PDN (IT)	12mg/30mg/10mg	5
Maintenance[c]		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly

[c] Patients in CR after protocol II or after the last R3 bloc for those qualified as VHR were eligible for the third randomization to test the advantage of adding monthly intravenous 6-mercaptopurine (1 g/m^2) to conventional maintenance therapy.

2.4 Protocol 58951

Description

From 1998 to 2008 patients with ALL were included in the 58951 protocol. Based on previous evidence obtained from preceding EORTC trials and other groups, the CLG decided to run this randomized trial to test the efficacy of dexamethasone vs prednisolone administered during induction regarding the EFS and survival in children with ALL and with lymphoblastic NHL and to assess the value of the increase of the number of administrations of L-Asparaginase during consolidation (protocol I) and during late intensification (protocol II) and the value of pulse of vincristine and prednisone during maintenance in average risk patients.

Objectives of the trial

- To assess the value of dexamethasone vs prednisolone administered during induction regarding the EFS and Survival in children with ALL and with lymphoblastic NHL.
- To assess the value of the increase of the number of administrations of L-Asparaginase during consolidation (protocol I) and during late intensification (protocol II) regarding the EFS and Survival in children without VHR features.
- To asses the value of pulse of vincristine and prednisone during maintenance in average risk patients.

Eligibility criteria

Inclusion criteria:

- Age less than 18 years.
- Previously untreated.
- Acute lymphoblastic leukemia of FAB L1 or L2 morphology whatever the immuno-phenotype or precursor B- and precursor T-lymphoblastic non-Hodgkin lymphoma.

Exclusion criteria:

- Acute lymphoblastic leukemia of FAB L3 morphology.
- Diffuse large cell B-cell lymphoma, Burkitt's lymphoma and high-grade B-cell lymphoma Burkitt like, according tot the REAL classification.

Eligibility criteria for the randomized trial pertaining to the corticoid question

Inclusion criteria:

- Fulfill all the inclusion and exclusion criteria mentioned before.

- Having been signed the informed consent before day 1 of the pre-phase or at the beginning or protocol IA.

Eligibility criteria for the randomized trial pertaining to the asparaginase question

Inclusion criteria:

- Achievement of complete remission or good partial response (GPR) for T-ALL between day 28 and day 42 of protocol I.
- Absence of VHR features.
- Absence of severe toxicity possibly related to asparaginase.

Eligibility criteria for the randomized trial pertaining to the pulses question

Inclusion criteria:

- Average risk patients
- Continuous complete remission at the time of maintenance.

Randomization

- This process was done centrally (EORTC Data Center, Brussels) by telephone or internet.

Risk groups

Very low risk (VLR)

1. ALL of B-cell lineage
2. and leukocyte count < $10x10^9$/l
3. and DNA index (DI) > 1.16 and < 1.50, and chromosome number 51-66 or DI > 1.16 and < 1.50, and chromosome number is unknown or chromosome number 51-66, and DI is not assessed
4. and good response to the prephase
5. and absence of t(9;22) / BCR-ABL, of t(4;11) / MLL-AF4, of 11q23 / MLL rearrangement and of AUL
6. and absence of CNS and gonadal involvement

Average risk (AR)

1. ALL with good response to the prephase who are neither VLR nor VHR
2. VLR ALL with CNS involvement (CSF+ or CSF +/-)
3. B lymphoblastic NHL stage III and IV without any VHR feature
4. T lymphoblastic NHL

AR patients are sub stratified in:

AR1:

- B-cell lineage ALL with < $100x10^9$/l
- surreptitious or hemorrhagic CSF becoming CSF negative at D4 of prephase

- precursor B lymphoblastic NHL stage III and IV
- precursor T lymphoblastic NHL stage I and II

AR2:

- B-cell lineage ALL with > $100x10^9$/l
- T-cell lineage ALL whatever the leukocyte count
- patients with overt or non-equivocal CNS involvement at D0 or any CSF involvement at D4
- gonadal involvement
- precursor T lymphoblastic NHL stage III and IV

Very high risk (VHR)

1. Poor response to the prephase (i.e. $\geq 1x10^9$/l blasts in peripheral blood after completion of the prephase)
2. or t(9;22) or bcr/abl
3. or t(4;11) / MLL-AF4
4. or 11q23 / MLL rearrangement
5. or near-haploidy (< 34 chromosomes or D.I. < 0.7)
6. or hypodiploid (35-40 chromosomes or D. I. > 0.7 and < 0.8)
7. or AUL
8. or for B-lineage ALL: failure to achieve CR after completion of protocol IA.
9. or for T-lineage ALL: failure to achieve CR or good partial response after completion ofprotocol IA
10. or minimal residual disease (MRD) > 10^{-2} (more than 1,000 blasts / 100,000 mononuclear BM cells) at evaluation of IA (on day 35)

Treatment

The protocol was a Berlin-Frankfurt-Munster (BFM)-like protocol, without cranial or local irradiation. Concerning the randomization dexamethasone *versus* prednisolone, the patients could be randomly assigned either before the beginning of the prephase (day 1), or at the beginning of protocol IA (day 8), at the investigator's discretion. In the latter case, prednisolone was used throughout the prophase.

All patients had to receive dexamethasone (6 mg/m^2/day) or prednisolone (60 mg/m^2/day), orally, in two divided doses throughout prephase (day 1 to day 7) and induction therapy (day 8 to day 35, including a tapering down period of 8 days). During protocol IIA, all patients received dexamethasone 6 mg/m^2.

If they had an HLA identical donor, all very high-risk patients were eligible for hematopoietic stem cell transplantation except those whose only very high-risk criterion was a poor corticosteroid response on day 8, without T-cell immunophenotype or early precursor B-

ALL or white blood cell count ≥100×10^9/L. Otherwise, the patients continued chemotherapy for a total treatment duration of 2 years.

Non VHR patients achieving acomplete remission or good partial response for T-ALL between day 28 and day 42 of protocol I were eligible for the second randomization:

a) Arm S=Short: no asparaginase during Ib and 4 injections of asparaginase during IIA

b) Arm L=Long: 8 injections of asparaginase during Ib and 8 during IIA

Average risk patients were eligible for the third randomization at the time of maintenance to receive or not pulses with vincristine and prednisone. Maintenance was prolonged for up to 2 years.

Representative schemas of the trial are shown in Figures 9 and 10.

Treatment details are depicted in tables 8, 9, 10 and 11.

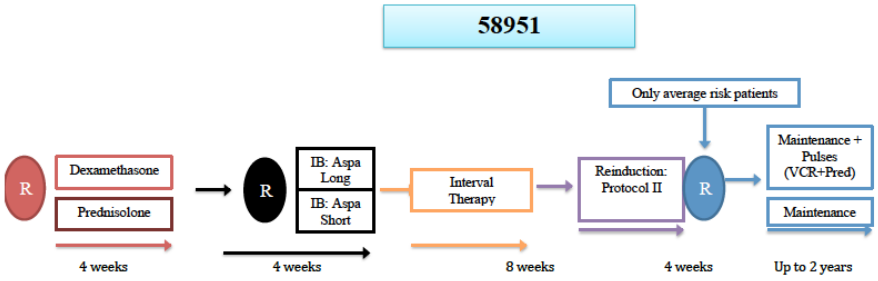


Figure 9. Trial 58951 schema

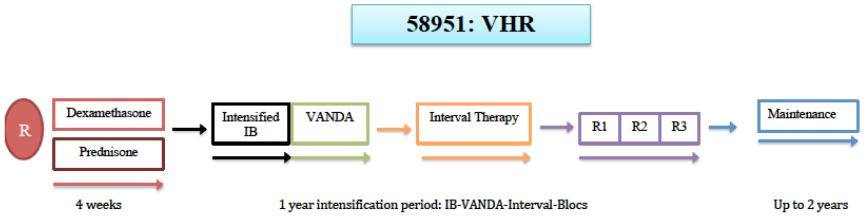


Figure 10. Trial 58951 VHR schema

Table 8 - EORTC-CLG 58951: Treatment protocol for very low risk (SR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	8-28
or Dexametasone	6mg/m^2	8-28
- Vincristine	1.5mg/m^2	8,15,22,29
- Daunorubicin	30mg/m^2	8,15
- Asparaginase (IV/IM)	10000IU/m^2	12,15,18,22,25,29,32,35
- Triple intrathecal chemo	Age dependent	12, 15
Protocol IB		
- Cyclophosphamide	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Triple intrathecal chemo	Age dependent	38, 52
- Asparaginase (IV/IM) (According to randomization)	0 or 5000UI/^{2}	38, 41, 45, 48, 52, 55, 59, 62
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate (24 h infusion)	5g/m^2	8,22,36,50
- Triple intrathecal chemo	Age dependent	9,23,37,51
Reinduction: Protocol II		
- Dexamethasone	6mg/m^2	1-21
- Vincristine	1.5mg/m^2	8,15,22,29
- Doxorubicin	30mg/m^2	8,15
- Asparaginase (IV/IM)	10000 IU/m^2	8,11,15,18
- Asparaginase (IV/IM) (According to randomization)	10000-5000 IU/m^2 Age dependent	8,11,15,18, 22, 25, 29, 32 38
- Triple intrathecal chemo	75mg/m^2	38-41, 45-48
- Cytarabine	60mg/m^2	36-49
- 6-Tioguanine		
Maintenance		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly

IT: Intrathecal**; IM:** Intramuscular; **IV:** Intravenous; **PO:** Per os

Table 9- EORTC-CLG 58951: Treatment protocol for average risk-1 (AR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	8-28
or Dexametasone	6mg/m^2	8-28
- Vincristine	1.5mg/m^2	8,15,22,29
- Daunorubicin	30mg/m^2	8,15, 22, 29
- Asparaginase (IV/IM)	10000IU/m^2	12,15,18,22,25,29,32,35
- Triple intrathecal chemo	Age dependent	12, 15
Protocol IB		
- Cyclophosphamide	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Triple intrathecal chemo	Age dependent	38, 52
- Asparaginase (IV/IM) (According to randomization)	0 or 5000UI/^{2}	38, 41, 45, 48, 52, 55, 59, 62
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate (24 h infusion)	5g/m^2	8,22,36,50
- Triple intrathecal chemo	Age dependent	9,23,37,51
Reinduction: Protocol II		
- Dexamethasone	6mg/m^2	1-21
- Vincristine	1.5mg/m^2	8,15,22,29
- Doxorubicin	30mg/m^2	8,15, 22, 29
- Asparaginase (IV/IM)	10000 IU/m^2	8,11,15,18
- Asparaginase (IV/IM) (According to randomization)	10000-5000 IU/m^2 Age dependent	8,11,15,18, 22, 25, 29, 32
- Cyclophosphamide	1g/m^2	36
- Triple intrathecal chemo	Age dependent	38
- Cytarabine	75mg/m^2	38-41, 45-48
- 6-Tioguanine	60mg/m^2	36-49
Maintenance [a]		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly
- Triple intrathecal chemo	Age dependent	Every 70 days (6 times)

IT: Intrathecal**; IM:** Intramuscular; **IV:** Intravenous; **PO:** Per os

[a] Average risk patients were eligible for the third randomization at the time of maintenance to receive or not pulses with vincristine and prednisone

Table 10- EORTC-CLG 58951: Treatment protocol for average risk-2 (AR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	8-28
or Dexametasone	6mg/m^2	8-28
- Vincristine	1.5mg/m^2	8,15,22,29
- Daunorubicin	30mg/m^2	15, 22, 29
- Asparaginase (IV/IM)	10000IU/m^2	12,15,18,22,25,29,32,35
- HD-MTX	5g/m^2	8
- Cyclophosphamide	1g/m^2	9
- Triple intrathecal chemo	Age dependent	12, 15
Protocol IB		
- Cyclophosphamide	1g/m^2	36,63
- Cytarabine	75mg/m^2	38-41, 45-48, 52-55, 59-62
- 6-Mercaptopurine	60mg/m^2	36-63
- Triple intrathecal chemo	Age dependent	38, 52
- Asparaginase (IV/IM) (According to randomization)	0 or 5000UI/^{2}	38, 41, 45, 48, 52, 55, 59, 62
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-56
- Methotrexate (24 h infusion)	5g/m^2	8,22,36,50
- Triple intrathecal chemo	Age dependent	9,23,37,51
Reinduction: Protocol II		
- Dexamethasone	6mg/m^2	1-21
- Vincristine	1.5mg/m^2	8,15,22,29
- Doxorubicin	30mg/m^2	8,15, 22, 29
- Asparaginase (IV/IM)	10000 IU/m^2	8,11,15,18
- Asparaginase (IV/IM) (According to randomization)	10000-5000 IU/m^2 Age dependent	8,11,15,18, 22, 25, 29, 32
- Cyclophosphamide	1g/m^2	36
- Triple intrathecal chemo	Age dependent	38
- Cytarabine	75mg/m^2	38-41, 45-48
- 6-Tioguanine	60mg/m^2	36-49
Maintenance[a]		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly
- Triple intrathecal chemo	Age dependent	Every 70 days (6 times)
- HD-MTX	5g/m^2	Every 70 days (6 times)
- Asparaginase	25000 IU/m^2	Every 70 days (6 times)

IT: Intrathecal**; IM:** Intramuscular; **IV:** Intravenous; **PO:** Per os

[a] Average risk patients were eligible for the third randomization at the time of maintenance to receive or not pulses with vincristine and prednisone

Table 11- EORTC-CLG 58951: Treatment protocol for very high risk (VHR)		
Treatment phase/drug	**Dose**	**Days Given**
Induction-Consolidation		
Protocol IA		
- Prednisolone	60mg/m^2	8-28
or Dexametasone	6mg/m^2	8-28
- Vincristine	1.5mg/m^2	8,15,22,29
- Daunorubicin	30mg/m^2	15, 22, 29
- Asparaginase (IV/IM)	10000IU/m^2	12,15,18,22,25,29,32,35
- HD-MTX	5g/m^2	8
- Cyclophosphamide	1g/m^2	9
- Triple intrathecal chemo	Age dependent	12, 15
Protocol IB		
- Dexamethasone	10mg/m^2	36-40, 50-54
- Vincristine	1.5mg/m^2	36, 41
- HD-MTX	5g/m^2	36, 50
- Cyclophosphamide	500mg/m^2	52, 53
- Cytarabine	2g/m^2/12h	40
- 6-Mercaptopurine	100mg/m^2	36-40
- 6-Tioguanine	100mg/m^2	50-54
- Triple intrathecal chemo	Age dependent	37, 51
- Daunorubicin	50mg/m^2	54
- Vindesine	3mg/m^2	50
- Asparaginase (IV/IM)	10000UI/^{2}	41, 43, 45, 55, 57, 59
VANDA		
- Dexamethasone	20mg/m^2	1-5
- Cytarabine	2g/m^2	1,2
- Mitoxantrone	8mg/m^2	3,4
- Etoposide	150mg/m^2	3,4,5
- Asparaginase	10000mg/m^2	7,9,11,13
- Methotrexate (IT)	12mg (age dependent)	5
Interval Therapy		
- 6-Mercaptopurine	25mg/m^2	1-42
- Methotrexate (24 h infusion)	5g/m^2	8,22,36
- Triple intrathecal chemo	Age dependent	9,23,37
Block R1		
- Dexamethasone	20mg/m^2	1-5
- 6-Mercaptopurine	100mg/m^2	1-5
- Vincristine	1.5mg/m^2	1,6
- Methotrexate	5mg/m^2	1
- Cytarabine	2mg/m^2	5
- Asparaginase	25000 IU/m^2	6
- MTX/Cytarabine/PDN (IT)	12mg/30mg/10mg	1

Block R2		
- Dexamethasone	20mg/m^2	1-5
- 6-Thioguanine	100mg/m^2	1-5
- Vindesine	3mg/m^2	1
- Methotrexate	5mg/m^2	1
- Ifosfamide	400mg/m^2	1-5
- Daunorubicin	50mg/m^2	5
- Asparaginase	25000mg/m^2	6
- Triple intrathecal chemo	Age dependent	1
Block R3		
- Dexamethasone	20mg/m^2	1-5
- Cytarabine	2g/m^2	1,2
- Etoposide	150mg/m^2	3,4,5
- Asparaginase	25000IU/m^2	6
- Triple intrathecal chemo	Age dependent	5
Maintenance		
- 6-Mercaptopurine (PO)	50mg/m^2	Daily
- Methotrexate (PO)	20mg/m^2	Weekly

IT: Intrathecal**; IM:** Intramuscular; **IV:** Intravenous; **PO:** Per os

IV. RESULTS

1. RESULTS

The following diagram represents the number of patients initially eligible for this study, those who were excluded and those finally included (Figure 11):

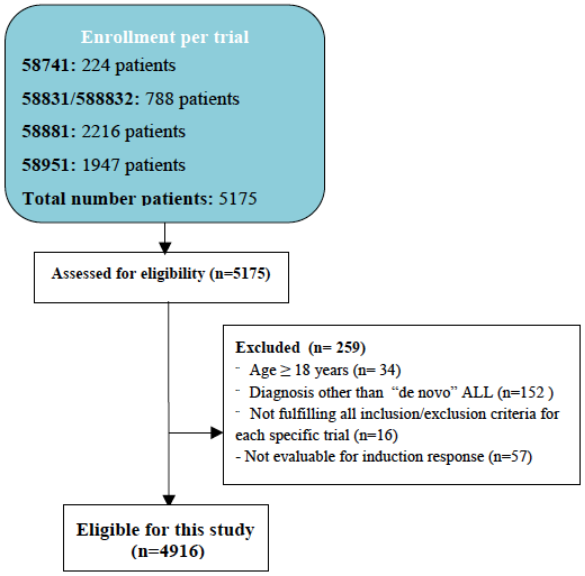


Figure 11. Consort diagram eligible patients

1.1 Protocol 58741

The trial was opened for recruitment in May 1971 and the last patient was entered in December 1978.

224 patients were initially recruited for this study.

10 patients were considered ineligible for the analysis according to the eligibility criteria specified in the protocol: Inadequate stage or histopathology at diagnosis (2), treatment never started (1), missing forms due to institutional difficulties (4), treatment stopped for more than 2 months (1), other protocol violations (2).

32 patients were not included for the analysis because they were ≥ 18 years at the time of diagnosis.

2 patients were ≥ 18 years at the time of diagnosis and also fulfilled any of the exclusion criteria.

2 patients were aged less than one year at the time of diagnosis but became older than one at the time of consolidation and therefore could be considered as eligible for the trial. These two patients were considered older than one year for the purposes of the analysis.

In the end, 40 patients were not included. That yielded 184 patients considered fully evaluable for the final analysis.

The numbers of patients randomized to each question proposed by the trial are described here:

- All 184 patients were randomized for the consolidation question to receive a polychemotherapy (P) (n=95, 51,6%) or a methotrexate (M) (n=89, 48,4%) based treatment.
- 110 patients were randomized for the maintenance question to receive chemotherapy (C) (n=54, 49,1%) or an immunotherapy (I) (n=56, 50,9%) based treatment. That was due to the fact that 51 patients relapsed during consolidation and 10 patients died in CR during this phase. Besides that, 5 patients refused further treatment, 5 violated the protocol during this phase, and in 3 patients the responsible physicians decided not to continue with the treatment due to excessive experienced toxicity. Nevertheless these patients were followed up and included in the survival analysis in order to comply with the intention to treat principle.

Baseline and demographic features of the 184 selected patients are depicted in table 12. Immunophenotype was not available for this trial.

Table 12- Baseline and demographical features of the 184 patients (%) - 58741 Trial	
Gender	
▪ **Male**	109 (59)
▪ **Female**	75 (41)
Median age at diagnosis (Years)	5 (1-18)
Age groups at diagnosis (Years)	
▪ **< 1**	0 (0)
▪ **1**	11 (5.9)
▪ **2 -5**	89 (48.1)
▪ **6 - 9**	48 (26)
▪ **≥ 10**	36 (20)
ALL immunophenotype	Not available in this trial
Risk groups	Not available in this trial
NCI risk group	
▪ **Standard**	119 (64.7)
▪ **High-risk**	65 (35.3)
CNS involvement	
▪ **Not involved**	177 (96.2)
▪ **Involved**	7 (3.8)
WBC groups at diagnosis ($x10^9$/l)	
▪ **< 10**	88 (47.6)
▪ **< 25**	30 (16.3)
▪ **< 100**	40 (21.6)
▪ **< 250**	13 (7)
▪ **≥ 250**	13 (7)

CNS: Central nervous system; **NCI:** National Cancer Institute; **WBC:** White blood cells. No immunophenotype data were available for this study.

Median follow up for the whole population was 9.4 years (0.2-17.3).

Median follow up for those patients who were still alive at last evaluation was 12.8 years (2.7-17). Last entry for one patient was 16 months after having started maintenance therapy. He was alive at that time and censored as alive at last follow up (2.7 years of follow up). If we omit this patient, median follow up for those patients who were still alive at last evaluation was 12.8 years (9.2-17).

Outcome of patients is depicted in table 13. At the time of the last evaluation 78 patients were still alive and in continual CR.

The EFS rate (±1 s.e.) was 77% ± 3.2% at 1 year, 44% ± 3.7% at 5 years and 43% ± 3.7% at 10 years.

The OS rates (±1 s.e.) were 88% ± 2.4% at 1 year, 55% ± 3.7% at 5 years and 49% ± 3.7% at 10 years.

Table 13 - Outcome of the 184 patients (%) - 58741 Trial	
Continuous CR[a]	78 (42.2)
Death in first CR	15 (8.1)
Relapse	91 (49.5)
Type of relapse	
▪ BM only	56 (30.4)
▪ CNS only	18 (9.8)
▪ Gonads only	6 (3.3)
▪ CNS combined[b]	8 (4.3)
▪ Others[c]	3 (1.6)

BM: Bone marrow; **CNS:** Central nervous system; **CR:** Complete remission
[a] **Continuous CR:** Includes only patients who remain in first CR after protocol first line therapy
[b] **CNS combined included:** 7 CNS and BM relapses and 1 CNS, BM and testicular relapse
[c] **Others included:** 2 combined BM and testicular relapses and 1 mediastinal relapse

91 patients experienced relapse. Median time to relapse was 15.7 months (0.9-123.6).

Of those 92 patients who experienced relapse, 79 eventually died. 12 of those 92 patients are still alive and in CR after subsequent treatment lines.

Median time to death for the whole population was 28 months (2.5-151.6).

A total of 15 patients died in first CR. 10 patients died during consolidation, 4 during maintenance and 1 after having completed treatment. Clinical features and causes of death for these patients according to the different randomized treatments are depicted in table 14.

Median time to death for those patients who died in first CR was 7.8 months (2.5-151.6).

Most frequent cause of death among those who died in first CR was infection (11 patients, 73.3%). Four patients died of *Pneumocystis jirovecii* infection, three during consolidation and two during maintenance. Four other patients died of disseminated varicella, representing all viral causes of death, two during consolidation and two during maintenance. *E. Coli* and *Klebsiella* were responsible in two other cases. In one patient, the infective organism could not be identified.

In three patients chemotherapy induced hepatic toxicity was the last cause of death, two during consolidation and one in maintenance.

One patient died of an unspecified brain tumor 12.5 years (151.6 months) after first diagnosis of ALL and 8 years after having completed all treatment respectively, including CNS irradiation. No toxic deaths happened during the immunotherapy maintenance phase.

Those 13 patients who did not proceed to the maintenance randomization due to toxicity, physicians' decision or protocol violation were still alive and in CR at last follow-up.

Table 14 - Clinical features and causes of death of the 15 patients who died in first complete remission (%) - 58741 Trial	
Gender	
▪ **Male**	9 (59.8)
▪ **Female**	6 (40.2)
Age groups at diagnosis (Years)	
▪ **< 1**	0 (0)
▪ **1**	3 (20)
▪ **2 -5**	10 (67.6)
▪ **6 - 9**	1 (6.7)
▪ **≥ 10**	1 (6.7)
ALL immunophenotype	Not available in this trial
Risk groups	Not available in this trial
NCI risk group	
▪ **Standard**	12 (79.9)
▪ **High-risk**	3 (20.1)
Death period	
▪ **P consolidation**	6 (40)
▪ **M consolidation**	4 (26.7)
▪ **P maintenance**	4 (26.7)
▪ **M maintenance**	0 (0)
▪ **Post – treatment**	1 (6.7)
Cause of death	
▪ **Infection**	11 (73.3)
• Bacterial	2
• Viral	4
• Fungal	0
• *Pneumocystis jirovecii*	4
• Unknown	1
▪ **Bleeding/Thrombosis**	0
▪ **Organ toxicity**	3 (20)
▪ **Transplant related mortality**	0
▪ **Secondary neoplasms**	1 (6.7)
▪ **Others**	0

NCI: National Cancer Institute

1.2 Protocols 58831/58832

These trials were opened for recruitment in July 1983 and the last patient was entered in June 1989.

Patients included in both studies will be presented together in this section.

788 patients were initially included in these trials.

8 patients had the diagnosis of NHL and therefore excluded from this analysis.

780 patients had the diagnosis of ALL. Among them, 735 were eligible for the evaluation of the induction response, whereas 45 were considered as ineligible: CNS involvement (7), suspicious CNS involvement in addition to other ineligibility criteria (2), unknown RF (2), response to treatment not evaluable (3), other reasons (31). Among the 735 eligible and evaluable patients, 11 were considered *a posteriori* by the study coordinator as having CNS involvement and 8 as a dubious or surreptitious involvement. However, they were included by the local investigators and therefore included in the analysis in order to comply with the intention to treat principle.

The numbers of patients randomized to each question proposed by the trial are described here:

- A total of 383 standard risk patients were randomized into two groups, to receive (n=192) or not (n=191) cyclophosphamide during IB.
- For medium and high-risk patients, a total of 183 patients were randomized into two groups, to receive high-doses methotrexate alone (n=90) or high doses methotrexate followed by cranial radiotherapy (n=93). The groups were well balanced regarding several initial features.

Baseline and demographic features of the 735 selected patients are depicted in table 15.

332 patients received any sort of pre-phase therapy before induction treatment. That was permitted by the protocol as long as it did not last for more than 8 days and treatment was limited to corticoids and/or vincristine. That was the case in all these patients. The reasons for prephase in this group were: leukocytosis (110), bulky disease (22), both (63) and others (137). 289 patients received exclusively steroids and 43 a combination of steroids and vincristine.

Table 15 - Baseline and demographical features of the 735 patients – 58831/58332 Trial	
Gender	
▪ **Male**	397 (54)
▪ **Female**	338 (46)
Median age at diagnosis (Years)	4.7 (0.1-17.7)
Age groups at diagnosis (Years)	
▪ **< 1**	23 (3.1)
▪ **1**	64 (8.7)
▪ **2 -5**	368 (50.1)
▪ **6 - 9**	152 (20.7)
▪ **≥ 10**	128 (17.4)
ALL immunophenotype	
▪ **B-Lineage**	547 (74.4)
▪ **T-Lineage**	94 (12.8)
▪ **Not available**	94 (12.8)
Risk groups	
▪ **SR**	463 (63)
▪ **MR/HR**	267 (36.3)
NCI Risk group[a]	
▪ **Standard**	484 (65.9)
▪ **High-risk**	250 (34.1)
CNS involvement[b]	
▪ **Unknown**	0 (0)
▪ **No (CNS-1)**	716 (97.4)
▪ **Dubious**	8 (1.1)
▪ **CNS-2**	0 (0)
▪ **CNS-3**	11 (1.5)
WBC groups at diagnosis ($x10^9$/l)[a]	
▪ **< 10**	326 (44.4)
▪ **< 25**	161 (21.9)
▪ **< 100**	170 (23.1)
▪ **< 250**	48 (6.5)
▪ **≥ 250**	29 (3.9)

CNS: Central nervous system; **HR:** High-risk**; MR:** Medium Risk**; NCI:** National Cancer Institute; **SR:** Standard risk**; WBC:** White blood cells

[a] One patient with an unknown WBC at diagnosis has not been included in the table

[b] 19 patients were found a posteriori to have CNS involvement despite the trial was designed for patients without CNS involvement at diagnosis.

A total of 707 (96.2%) patients achieved complete remission after induction.

28 patients did not achieve a CR at the end of induction/consolidation or died before the response to treatment could be evaluated.

The EFS rate (±1 s.e.) was 92% ± 1.0% at 1 year, 70% ± 1.7% at 5 years, 68% ± 1.8% at 10 years and the estimated OS rates (±1 s.e.) were 94% ± 0.9% at 1 year, 80% ± 1.5% at 5 years and 77% ± 1.6% at 10 years.

Outcome of patients is depicted in table 16.

Table 16- Outcome of the 735 patients (%) – 58831/58332 Trial	
No CR after induction/consolidation[a]	28 (3.8)
▪ Induction failure	12 (1.6)
▪ Early death	16 (2.2)
Continuous CR[b]	480 (65.3)
Death in first CR	9 (1.2)
Relapse	217 (29.6)
Type of relapse	
▪ **BM only**	112 (15.3)
▪ **CNS only**	42 (5.7)
▪ **Gonads only**	8 (1.1)
▪ **CNS combined**[c]	31 (4.2)
▪ **Others**[d]	24 (3.2)

BM: Bone marrow; **CNS:** Central nervous system **CR:** Complete remission;

[a] Including the 16 patients who died before they could be evaluated for response after induction/consolidation

[b] **Continuous CR:** Includes only patients who remain in first CR after protocol first line therapy

[c] **CNS combined included:** 27 combined CNS and BM, 2 CNS and gonadal and 2 CNS, BM and gonadal.

[d] **Others included:** 14 combined BM and gonadal, 2 BM and mediastinal, 1 BM and bone, 2 BM and ganglionar, 1 testicular and ocular, 1 ganglionar, 1 bone and 1 ocular relapse. In 2 cases the data

12 out of these 28 patients not achieving a CR after induction could be evaluated. In 4 patients no evidence of response was observed and they were considered as absolutely resistant at the end of the consolidation. In 6 patients the best response achieved was a partial response (PR) at the end of consolidation. 1 patient persisted in hypoplasia for more than four weeks after having completed the consolidation and 1 patient presented still extra medullary disease at the end of the consolidation; therefore, both patients were considered no responders to induction/consolidation therapy. 2 of these patients continued therapy according to protocol but considered as failures and the other 10 underwent second line therapies according to responsible physicians' decisions including 3 allogenic and 2 autologous transplants. At last follow up 5 patients were still alive in CR.

16 out of these 28 patients died before having obtained a conclusive response to induction therapy and were considered as having died before a first CR was achieved.

Two patients died before starting any anticancer therapy in the context of very-high WBC (475 and 489 $x10^9$/l). One patient died before leukapheresis could be started and died of DIC and one other died once leukapheresis was started but he did not respond and died of multi-organ failure.

During induction 9 patients died before they could be evaluated for response and 5 other patients were found to have hypoplastic bone marrow after induction and died before proceeding to consolidation. 6 of these 14 patients were given any sort of pre-phase treatment (4 due to leukocytosis, 1 due to leukocytosis and bulky disease and 1 for other reason). Clinical features and causes of death for these patients are pictured in table 17.

Most frequent type of death in this group of 16 patients was infection (11 patients, 68.8%). Bacterial organisms represented the majority of infectious related deaths, mainly *Streptococcus pyogenes* (4) and P*seudomonas* (3); one patient died of a septic shock of probably urinary origin by *E. Coli* and *Klebsiella*. In second place fungal organisms, two pulmonary and one cerebral aspergillosis. Five patients died of bleeding complications, mainly intracranial hemorrhage (3) and DIC (2). In one of the patients with cerebral hemorrhage there was a pulmonary aspergillosis associated and in one patient with DIC there was a sepsis by *Streptococcus pyogenes* associated. One patient with a pulmonary aspergillosis experienced also necrotizing enterocolitis (‘other’ in table). One patient with congenital undifferentiated ALL presented with very high WBC (960 $x10^9$/l). He died on day 48 after having completed IA but whose response could not be evaluated and was considered of having died of disease in the absence of any other reasonable cause of death.

Table 17- Clinical features and causes of death of the 16 patients who died before getting into complete remission (Early Deaths) (%) - 58831/2 Trials	
Gender	
▪ **Male**	6 (37.5)
▪ **Female**	10 (62.5)
Time period of death	
▪ **Death before treatment**	2 (12%)
▪ **Death during pre-phase**	0 (0%)
▪ **Death during induction**	9 (88%)
Age groups at diagnosis (Years)	
▪ **< 1**	1 (6.3)
▪ **1**	1 (6.3)
▪ **2 -5**	7 (44.1)
▪ **6 - 9**	3 (18.9)
▪ **≥ 10**	4 (25.2)
WBC groups at diagnosis ($x10^9$/l)	
▪ **< 10**	9 (55.8)
▪ **< 25**	2 (12.6)
▪ **< 100**	1 (6.3)
▪ **< 250**	1 (6.3)
▪ **≥ 250**	3 (18.9)
ALL immunophenotype	
▪ **B-Lineage**	12 (75.8)
▪ **T-Lineage**	2 (12.6)
▪ **Not available**	2 (12.6)
NCI Risk group	
▪ **Standard**	8 (50)
▪ **High-risk**	8 (50)
Cause of death	
▪ **Tumour burden**	2 (10)
▪ **Infection**	11 (55)
• Bacterial[a]	8
• Viral	0
• Fungal	3
• *Pneumocystis jirovecii*	0
• Others	0
▪ **Bleeding/Thrombosis**[b]	5/0 (25)
▪ **ALL progression**	1 (5)
▪ **Others**[c]	1 (5)

NCI: National Cancer Institute

[a] One patient died of a sepsis of urinary origin by *E. Coli* and *Klebsiella*

[b] One patient died of pulmonary aspergillosis and cerebral hemorrhage; another one died of *Streptococcus pyogenes* sepsis and DIC. These 2 patients have been categorized in the infection group and bleeding group. One patient died of DIC in the context of hyperleukocytosis and he has been categorized in the group of bleeding and tumor burden.

[c] One patient died of necrotizing enterocolitis and had pulmonary aspergillosis

Nine patients received an HSCT in first CR as consolidation therapy between September 1986 and November 1989. 8 patients had an allogeneic HSCT while only one had an autologous. 3 of these patients died afterwards. 2 patients receiving an allograft died of causes related to the transplant as described below and the patient receiving the auto graft relapsed in the marrow eleven months later and subsequently died of disease.

Nine patients died in first CR at some point during treatment. Median time to death for this group of patients was 0.7 years (0.2-2.2). These patients are displayed in table 18.

Table 18 - Clinical features and causes of death of the 9 patients who died in first complete remission (%) – 58831/2 Trials	
Gender	
▪ **Male**	7 (77.8)
▪ **Female**	2 (22.2)
Age groups at diagnosis (Years)	
▪ **< 1**	0 (0)
▪ **1**	1 (11.1)
▪ **2 -5**	6 (66.6)
▪ **6 - 9**	0 (0)
▪ **≥ 10**	2 (22.2)
ALL immunophenotype	
▪ **B-Lineage**	5 (55.5)
▪ **T-Lineage**	2 (22.2)
▪ **Not available**	2 (22.2)
Risk groups	
▪ **Standard Risk**	5 (55.5)
▪ **Medium Risk**	3 (33.3)
▪ **High-Risk**	1 (11.1)
NCI Risk group	
▪ **Standard**	6 (66.6)
▪ **High-risk**	3 (33.4)
Death period	
▪ **Interval therapy**	2 (22.2)
▪ **Protocol II**	1 (11.1)
▪ **HSCT**	2 (22.2)
▪ **Maintenance**	4 (44.4)
▪ **Post – treatment**	0 (0)
Cause of death	
▪ **Infection**	4 (44.4)
• Bacterial	0
• Viral	3
• Fungal	1
• *Pneumocystis jirovecii*	0
• Others	0
▪ **Bleeding/Thrombosis**	0 (0)
▪ **Organ toxicity**	0 (0)
▪ **Transplant related**	2 (22.3)
▪ **Secondary neoplasms**	0 (0)
▪ **Others**	3 (33.3)

HSCT: Hematopoietic stem cell transplantation; **NCI:** National Cancer Institute; **WBC:** White blood cell(s)

Most frequent cause of death among these 9 patients was infection (4 patients, 44.4%). Viral infections accounted for the majority of events (3), two measles induced pneumopathy and one ECHO virus 6 encephalopathy and pneumopathy. All of them occurred during maintenance. Pulmonary aspergillosis resulted in death in one patient during Protocol II.

Two of these patients died after having received an allogenic transplant in first CR meaning for a TRM of 22.2% (2 cases of TRM in 9 patients who received a graft in first CR).

The first one was found to be positive for the Philadelphia chromosome at the end of the consolidation. For that reason, this patient was decided to receive a matched related allogeneic transplant after consolidation therapy. He died two months after transplant of conditioning regimen related toxicity.

The second patient received the interval and protocol therapies according to trial directions. He underwent a matched related allogeneic transplant after protocol II and died one month later of a capillary leak syndrome related to the conditioning regimen and cyclosporine toxicity.

Three patients form the category of others.

Two patients died of malignant systemic histiocytosis during treatment, one after interval therapy and one during maintenance. Presentation was similar in both cases with malaise, fevers, anemia, and splenomegaly. One patient presented also bone, bone marrow and pleural invasion of histiocytes. No evidence of leukemic cells was found in any of the biological samples tested and infectious causes were thoroughly ruled out. Progression of the disease was extremely quick in first case. Second patient received a course of COPAD chemotherapy and achieved a CR but died of cardiac arrest days after the start of salvage treatment.

Only one patient died of a non-toxic cause. Last cause of death was assumed by the investigators to be a supraventricular tachycardia (SVT) related to the manipulation of the central venous catheter (CVC) during a flushing procedure. Autopsy was inconclusive suggesting the possibility of fat emboli.

1.3 Protocol 58881

This trial was opened for registration in January 1989 and the last patient was entered in November 1998.

2216 patients were initially included in this trial.

2077 had the diagnosis of ALL while 139 had the diagnosis of NHL and therefore not included in this study.

12 out of the 2077 ALL patients could not be evaluated for response due to lack of information (3), protocol violation (1), and treatment refusal (1) or other reasons (7). That yielded 2065 patients fully evaluable for this study.

The numbers of patients randomized to each question proposed by the trial are described here:

- 653 ALL children were randomized to receive *Escherichia Coli* asparaginase or Erwinia asparaginase
- 593 patients were randomized to two groups to test the value of cytarabine in interval therapy
- 820 patients were randomized into two groups for maintenance therapy without or with i.v. Mercaptopurine.

Baseline and demographic features of the 2065 selected patients are depicted in table 19.

Table 19 - Baseline and demographical features of the 2065 patients (%) – 58881 Trial	
Sex	
▪ **Male**	1163 (56.3)
▪ **Female**	902 (43.7)
Median age at diagnosis (Years)	4 (0-17)
Age groups at diagnosis (Years)	
▪ **< 1**	60 (2.9)
▪ **1**	160 (7.7)
▪ **2 -5**	1079 (52.3)
▪ **6 - 9**	405 (19.6)
▪ **≥ 10**	361 (17.5)
Immunophenotype	
▪ **B-Lineage**	1766 (85.5)
▪ **T-Lineage**	299 (14.5)
NCI Risk group [a]	
▪ **Standard**	1305 (63.2)
▪ **High-risk**	759 (36.8)
CNS involvement	
▪ **Unknown**	18 (0.9)
▪ **No (CNS-1)**	1889 (91.5)
▪ **Dubious**	53 (2.6)
▪ **CNS-2**	51 (2.5)
▪ **CNS-3**	54 (2.6)
WBC groups at diagnosis ($x10^9$/l)[a]	
▪ **< 10**	915 (44.3)
▪ **< 25**	384 (18.6)
▪ **< 100**	469 (22.7)
▪ **< 250**	167 (8.1)
▪ **≥ 250**	128 (6.2)

CNS: Central nervous system**; NCI:** National Cancer Institute; **WBC:** White blood cell(s)
[a] One patient with an unknown WBC at diagnosis, has not been included in the table.

32 ALL patients were Down syndrome.

Median follow up for the whole population was 7.4 years (0-18.2).

Median follow up for those patients who were still alive at last evaluation was 8.3 years (1.3-18.2).

2019 (97.7%) patients achieved a CR at the end of induction/consolidation.

46 patients (1.8%) did not achieve a CR at the end of induction/consolidation or died before the response to treatment could be evaluated.

Outcome of patients is shown in table 20.

Table 20 - Outcome of the 2065 patients (%) – 58881 Trial	
No CR after induction/consolidation [a]	46 (2.2)
▪ **Induction failure**	27 (1.3)
▪ **Early death**	19 (0.9)
Continuous CR [b]	1443 (69.9)
Death in first CR	64 (3.1)
Relapse	510 (24.7)
Type of relapse	
▪ **BM only**	299 (14.5)
▪ **CNS only**	71 (3.4)
▪ **Gonads only**	24 (1.2)
▪ **CNS combined**	77 (3.7)
▪ **Others**	39 (1.9)

BM: Bone marrow; **CNS:** Central nervous system**; CR:** Complete remission

[a] Including the 19 patients who died who died before they could be evaluated for response after induction

[b] **Continuous CR:** Includes only patients who remain in first CR after protocol first line therapy

The overall EFS rate (±1 s.e.) was 94% ± 0.5% at 1 year, 75% ± 1% at 5 years, 73.3% ± 1% at 8 years and 73% ± 1.1% at 10 years and the estimated survival rates were 96% ± 0.4% at 1 year, 84% ± 0.8% at 5 years and 81% ± 0.9% at 10 years.

83 patients received a HSCT in first CR. Of those patients 79 had an allogeneic HSCT while 4 had an autologous.

Donor type for those who received an allo-HSCT was: matched sibling donor (MSD) (52), matched unrelated donor (MURD) (17), haploidentical (4), mismatched related donor (MMRD) (3), mismatched unrelated donor (MMURD) (1), cord blood (CB) (1) and not known in one patient.

20 ALL patients developed a secondary neoplasm during or after treatment, 16 in first CR, 2 in second CR and 2 in partial remission. They could be split among: acute myeloid leukemia (AML) (9), bone/soft tissues sarcomas (3), Hodgkin lymphoma (2), papillary thyroid carcinoma (2), B-Cell NHL (1), renal PNET (1), cutaneous histiocytosis (1) and peritoneal carcinomatosis (1).

19 patients died before getting into complete remission. These patients' characteristics are displayed in table 21.

Most frequent type of death among these 19 patients was infection (10 patients, 52.6%). Bacterial organisms represented the majority of infectious related deaths, mainly *Streptococcus*

(3) and P*seudomonas* (3), followed by fungal organisms (one pulmonary aspergillosis with associated sepsis by pseudomona), and others unknown but presumably bacterial (3). One of these patients was found to have an interstitial pneumonia. Despite all efforts made to identify the cause the patient died in the context of respiratory failure but the organism could not be identified.

Five patients died before any treatment could be initiated: 1 sepsis by *Streptococus*, 1 ARDS, 1 multi-organ failure in the context of a very high WBC (1.250 $x10^9$/l) and associated *Streptococcus* infection that did not respond to leukapheresis, 1 cerebral hemorrhage with high WBC count (580 $x10^9$/l) and 1 patient was considered to have died of progressive ALL before any anticancer therapy could be started

During induction, intracranial hemorrhage was responsible for death in 2 patients; one with a very high WBC count (600 $x10^9$/l). Two patients died of gastrointestinal bleeding during induction. Two other patients died in the context of an adult respiratory distress syndrome (ADRS) and veno-occlusive disease (VOD).

Table 21- Clinical features and causes of death of the 19 patients who died before getting into complete remission (Early Deaths) (%) - 58881	
Gender	
▪ **Male**	8 (42.1)
▪ **Female**	11 (57.9)
Time period of death	
▪ **Death before treatment**	5 (26.3)
▪ **Death during pre-phase**	5 (26.3)
▪ **Death during induction**	9 (47.4)
Age groups at diagnosis (Years)	
▪ **< 1**	7 (37.1)
▪ **1**	2 (10.6)
▪ **2 -5**	4 (21.2)
▪ **6 - 9**	1 (5.3)
▪ **≥ 10**	5 (26.5)
WBC groups at diagnosis (x10^9/l)	
▪ **< 10**	7 (37.1)
▪ **< 25**	0 (0)
▪ **< 100**	2 (10.6)
▪ **< 250**	4 (21.2)
▪ **≥ 250**	6 (31.8)
ALL immunophenotype	
▪ **B-Lineage**	17 (89.4)
▪ **T-Lineage**	2 (10.6)
▪ **Not available**	0 (0)
NCI Risk group	
▪ **Standard**	4 (21.2)
▪ **High-risk**	15 (78.8)
Cause of death	
▪ **Tumour burden** [a]	1 (5)
▪ **Infection**	10 (50)
• Bacterial	6
• Viral	0
• Fungal	1
• *Pneumocystis jirovecii*	0
• Unknown	3
▪ **Bleeding/Thrombosis**	5/0 (25)
▪ **ALL progression**	1 (5)
▪ **Others** [c]	3 (15)

NCI: National Cancer Institute; **WBC:** White blood cell(s)

[a] One patient died of leukostasis related complications and *Streptococcus* sepsis. He has been categorized in the tumour burden group and in the infection group.

[c] Others: 2 ARDS (one during pre-phase, one in induction) and 1 VOD.

A total of 64 patients died in CR and they are presented in table 22.

Infection was the most frequent cause of death in these 64 patients when considering also the infections leading to death after bone marrow transplant (21 during chemotherapy and 11 after HSCT respectively, 50%).

For those who did not undergo a bone marrow transplant, bacterial organisms represented the majority of infectious related deaths with no specific predominant organism: *E. Coli* (1), *Lysteria monocytogenes* (1), *Mycoplasma pneumoniae* (1), *Enterobacter* (1), *Staphylococus epidermidis*, and three bacterial organisms but not confirmed the specie.

Viruses were responsible for death in four cases, two measles induced pneumopathy, one secondary hemophagocytic lymphohistiocytosis (HLH) driven by EBV and one *Influenza B* induced myocardiopathy. Three events happened during maintenance and one during R2 block. *Aspergillus* was the most frequent fungal organism responsible for death in first CR (4) followed by *Candida* (2), mostly during consolidation therapy and one fungal infection but not confirmed the specie.

Two patients died of pulmonary embolism (PE). In one case it was related to the CVC recently inserted during phase IIA in a patient with congenital stenosis of pulmonary arteries but the autopsy confirmed that the embolism was not related to the cardiac abnormality. The other patient died of PE suspected of being of fungal origin but with un-identified organism.

Six patients were grouped in the category of death of organ toxicity. The assessment of relationship was made by the local investigators: one of anthracycline cardiac related toxicity (cumulative doses of Daunurubicin 120mg/m^2 and Doxorubicin 120mg/m^2), one of toxic epidermal toxicity (Lyell's syndrome), one of undefined hepatic toxicity and one of pancreatitis related to native *E. Coli* Asparaginase. One patient died of renal toxicity related to MTX and *Enterobacter Cloacae* sepsis and one of myocardiopathy related to anthracycline toxicity and *Influenza B* infection. This later patient was treated in the very high-risk group and had already received 220mg/m^2 of Daunorubicin and 16mg/m^2 of Mitoxantrone.

Six patients died of other causes: one undefined pulmonary disease in a patient with ataxia telangiectasia syndrome, one undefined neurological/cardiological disease, one primary HLH, one macrophage activation syndrome (MAS), one patient that experienced a cardiac arrest when was inserted a CVC in the context of a *Candida* sepsis and one sudden death at home.

Autopsy was not performed in this patient with unclear causes although last evaluation was normal. Five events happened while on treatment.

22 patients died of causes directly related to the bone marrow transplant procedure itself accounting for a TRM of 26.5% (22 cases of TRM in 83 patients who received a graft in first CR). In 12 cases the transplant was performed from a MSD, in 6 cases from a MURD, in 2 cases from a haploidentical donor, in 1 from a MMURD and in 1 from a mismatched CB donor.

Causes of death in this group could be split among: infection (11), GVHD (7), intracranial hemorrhages (2) and others (2).

Infection was the most frequent cause of death in patients receiving a transplant. Fungal infections were the most common causes of death after graft, responsible in 5 cases (4 *Aspergillus* and one *Candida*). Viruses, including a post-transplant limphoproliferative disorder-EBV mediated, one disseminated HSV and one *Papillomavirus* related leukoencephalopathy, were responsible for 3 deaths. Toxoplasmosis involving the brain and the lung was the cause of death in 2 patients respectively. In one patient the cause of death was a septic episode but the germ could not be identified.

Acute GVHD was the cause in 3 patients while chronic was in 4 patients. 4 of these patients had received the cells from a matched sibling donor while 3 from a MUD.

Intracranial hemorrhages were responsible of death in 2 patients.

Finally, one patient died of an undefined lung insufficiency and other developed an ARDS, the causes of which could not be identified.

11 ALL patients who achieved CR after first line therapy and developed a second neoplasm died of this second malignancy (10) or related toxicity (1). That was the case in one patient who developed a secondary AML and died days after of the bone marrow transplant while in CR for the AML due to conditioning regimen related toxicity. Only one patient who developed AML had received epipodophyllotoxins in first line.

Therefore, AML was responsible for death in 7 patients while the other 4 died from Ewing sarcoma, adult type sarcoma, peritoneal carcinomatosis and pulmonary epithelioma with cerebral involvement.

Table 22 - Clinical features and causes of death of the 64 patients who died in first complete remission (%) – 58881 Trial	
Gender	
▪ **Male**	36 (56.3)
▪ **Female**	28 (33.7)
Age groups at diagnosis (Years)	
▪ **< 1**	1 (1.6)
▪ **1**	3 (4.7)
▪ **2 -5**	19 (29.7.2)
▪ **6 - 9**	15 (23.4)
▪ **≥ 10**	26 (40.6)
ALL immunophenotype	
▪ **B-Lineage**	49 (76.6)
▪ **T-Lineage**	15 (23.4)
NCI Risk group	
▪ **Standard**	23 (35.9)
▪ **High-risk**	41 (64.1)
Death period	
▪ **Consolidation**	9 (14.1)
▪ **Interval therapy**	3 (4.7)
▪ **Protocol II**	10 (15.6)
▪ **Blocs**	2 (3.1)
▪ **HSCT**	22 (34.4)
▪ **Maintenance**	10 (15.6)
▪ **Post – treatment**	8 (12.5)
Cause of death	
▪ **Infection**	19 (30.9)
• Bacterial	8
• Viral	4
• Fungal [a]	7
• *Pneumocystis jirovecii*	0
• Others	0
▪ **Bleeding/Thrombosis** [b]	0/2 (2.9)
▪ **Organ toxicity** [c]	6 (8.7)
▪ **Transplant related**	22 (32.3)
▪ **Secondary neoplasms**	11 (16)
▪ **Others**	6 (8.8)

NCI: National Cancer Institute; **HSCT:** Hematopoietic stem cell transplantation

[a] One patient died of combined infection of *S. Aureus* and *Candida* sepsis

[b] One patient experienced PE related to a undefined fungal infection

[c] One patient died of Grade IV renal toxicity related to MTX and Enterobacter Cloacae sepsis and one patient died of a myocardiopathy related to anthracyclines and *Influenza B* infection. They have been categorized in the group of infection and therapy induced toxicity.

1.4 Protocol 58951

This trial was opened for registration in December 1998 and August 2008.

1947 patients with newly diagnosed ALL were enrolled in the EORTC 58951 trial. Nine were considered ineligible (6 had been already treated with steroids and 3 because they had another disease rather than ALL).

At the time of finishing collecting the data for this cohort (Year 2012), data was not mature enough and therefore the analysis of outcome and risk factors regarding toxic deaths in this population was not possible, as it has been done for previous protocols.

Therefore, only a descriptive analysis of toxic deaths for this trial will be performed.

98 patients underwent bone marrow transplantation in first complete remission.

14 patients died before achieving a first complete remission (0.7%) and 35 in first complete remission (1.8%).

Clinical features and causes of death for these patients are presented in tables 23 and 24.

In those who died before getting into remission, most frequent type of death was infection (13 patients, 92.8%). Bacterial organisms were represented by: *Enterobacter cloacae* (3), *Streptococus* B (2), *Proteus* (2), *Staphylococus haemoliticus* (1), *Pseudomonas* (2), *E. Coli* (3). In three cases there were two bacterial agents that were considered cause of infection leading to death. There were 3 patients who died from pulmonary invasive aspergillosis. One patient died of a gastrointestinal bleeding. In this patient a pulmonary co-infection by *Pseudomonas* was evidenced.

Table 23- Clinical features and causes of death of the 14 patients who died before getting into complete remission (Early Deaths) (%) - 58951	
Gender	
▪ **Male**	5 (35.7)
▪ **Female**	9 (66.3)
Time period of death	
▪ **Death before treatment**	0 (0)
▪ **Death during pre-phase**	0 (0)
▪ **Death during induction**	14 (100)
Age groups at diagnosis (Years)	
▪ **< 1**	0 (0)
▪ **1**	0 (0)
▪ **2 -5**	5 (35.7)
▪ **6 - 9**	5 (35.7)
▪ **≥ 10**	4 (28.5)
WBC groups at diagnosis (x10^9/l)	
▪ **< 10**	4 (28.5)
▪ **< 25**	4 (28.5)
▪ **< 100**	6 (43)
▪ **< 250**	0 (0)
▪ **≥ 250**	0 (0)
ALL immunophenotype	
▪ **B-Lineage**	10 (75)
▪ **T-Lineage**	4 (25)
▪ **Not available**	0
NCI Risk group	
▪ **Standard**	10 (71.4)
▪ **High-risk**	4 (28.6)
Cause of death	
▪ **Tumour burden**	0 (0)
▪ **Infection**	13 (92.8)
• Bacterial [a]	13
• Viral	0
• Fungal	3
• *Pneumocystis jirovecii*	0
• Others	0
▪ **Bleeding/Thrombosis**	1/0 (7.2)
▪ **ALL progression**	0 (0)
▪ **Others**	0 (0)

NCI: National Cancer Institute; **WBC:** White blood cell(s)
[a] Three patients experienced a combined bacterial infection

A total of 35 patients died in first complete remission. They are presented in table 24.

Infection was the most frequent cause of death in these patients when considering also the infections leading to death after bone marrow transplantation (16 patients during chemotherapy, and 4 in transplant patients).

Among those who received conventional chemotherapy, bacterial organisms represented the majority of infectious related deaths with no specific predominant microorganism: *E. Coli* (2), *Proteus* (1), *Streptotocus* (1), *Pseudomona* (1) and *Enterococus* (1). One patient developed a macrophage activation syndrome (MAS) related to *Flavimonas Oryzihabitans.* Two patients died of a sepsis of unknown origin (one of them with associated pulmonary hemorrhage and thrombosis).

Viruses were responsible in 2 cases (2 during chemo and 2 during HSCT). In those treated with chemo there were one VHB infection and one varicella pneumonia.

4 patients had fungal infections, 3 *Aspergillus* and 1 Candida, all in patients getting chemotherapy, during phases of intensive therapy (Protocol II and VANDA).

One patient in this trial died of a *Pneumocistiss Jiroveciii* lung infection. This patient was under prophylaxis for this germ, but despite this he developed this infection. This patient was treated with high doses but the infection progressed and finally died.

One patient had diffuse pulmonary and gastrointestinal hemorrhage.

In the group of others, two patient developed and ARDS, two MAS of unknown origin, one systemic vasulitis, one cardiomyopathy not related to anthracyclines and one accident after finishing treatment. One patient had a cardiogenic shock; the autopsy suggested that it may had been related to a viral infection but it could not finally confirmed.

Transplant related mortality in this cohort was lower than in the previous trial: 7 out of 98 patients transplanted in first remission died (TRM=7,1%). Among those patients who were transplanted:

- 4 died of infection: 1 PTLD-EBV driven, 1 systemic adenovirus with fulminant hepatitis and multi-organ failure (MOF), 1 VZV encephalitis and one bacterial infection non identified.
- 2 died of graft-versus host disease (1 acute and one chronic)
- 1 died of MOF of un-identified origin

3 patients developed a secondary malignancy leading to death: 2 AML, 1 meduloblastoma. One AML and the medulloblastoma occurred during maintenance therapy.

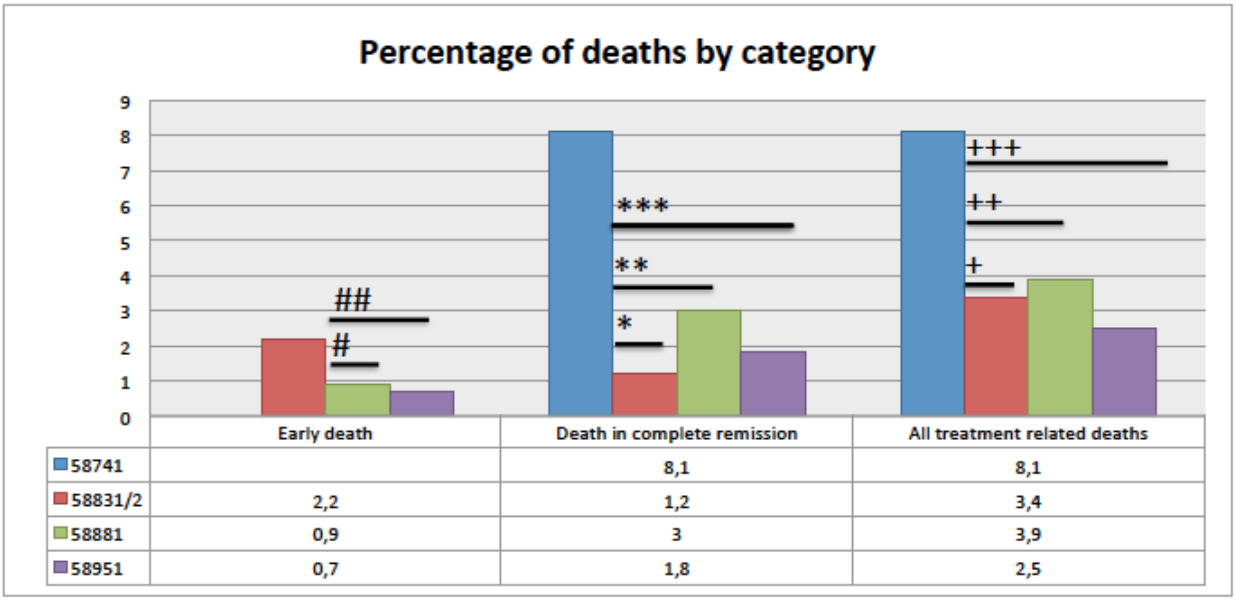
Table 24 - Clinical features and causes of death of the 35 patients who died in first complete remission (%) – 58951 Trial	
Gender	
▪ **Male**	21 (60)
▪ **Female**	14 (40)
Age groups at diagnosis (Years)	
▪ **< 1**	0 (0)
▪ **1**	2 (5.7)
▪ **2 -5**	13 (37.1)
▪ **6 - 9**	6 (17.1)
▪ **≥ 10**	14 (40)
ALL immunophenotype	
▪ **B-Lineage**	27 (77.1)
▪ **T-Lineage**	8 (22.9)
NCI Risk group	
▪ **Standard**	14 (40)
▪ **High-risk**	21 (60)
Death period	
▪ **Consolidation**	5 (14.3)
▪ **Interval therapy**	1 (2.9)
▪ **Protocol II**	7 (20)
▪ **Blocs**	3 (8.6)
▪ **VANDA**	6 (17.1)
▪ **HSCT**	7 (20)
▪ **Maintenance**	4 (11.4)
▪ **Post – treatment**	2 (5.7)
Cause of death	
▪ **Infection**	16 (44.4)
• Bacterial	7
• Viral	2
• Fungal	4
• *Pneumocystis jirovecii*	1
• Unknown	2
▪ **Bleeding/Thrombosis** [a]	1/1 (5.5)
▪ **Organ toxicity**	0 (0)
▪ **Transplant related**	7 (19.4)
▪ **Secondary neoplasms**	3 (8.3)
▪ **Others**	8 (22.2)

NCI: National Cancer Institute; **HSCT:** Hematopoietic stem cell transplantation

[a] One patient had sepsis of unknown origin and associated pulmonary thrombosis and hemorrhage and he has been included in the group of infection and thrombosis.

1.5 Summary of results

A significant reduction of all treatment related deaths has been observed between the 58741 protocol (8.1%) and the three other trials (3.4%, 3.9% and 2.5% respectively) (Figure 12).



	Early death	Death in complete remission	All treatment related deaths
58741		8,1	8,1
58831/2	2,2	1,2	3,4
58881	0,9	3	3,9
58951	0,7	1,8	2,5

Figure 12. Percentage of treatment related deaths by category

Early death

58831/2 Vs 58881: # $\chi 2$=1296.9; df=1; p=<0.001
58831/2 Vs 58951: ## $\chi 2$=1434.1; df=1; p=<0.001

Death in complete remission

58741 Vs 58831/2: * $\chi 2$=393; df=1; p=<0.001
58741 Vs 58881: ** $\chi 2$=556.1; df=1; p=<0.001
58741 Vs 58951: *** $\chi 2$=417.1; df=1; p=<0.001

All treatment related deaths

58741 Vs 58831/2: + $\chi 2$=243.9; df=1; p=<0.001
58741 Vs 58881: ++ $\chi 2$=658.9; df=1; p=<0.001
58741 Vs 58951: +++ $\chi 2$=944.5; df=1; p=<0.001

On average, the rate of toxic deaths in all four trials is 4.5%. If we exclude the 58741 trial that has the highest rate of toxicity and was performed early in the decade of the 70´s, the rate is 3.3%.

A detailed summary of infective and non-infective treatment related deaths by category, protocol and phase of protocol in Appendix 2.

1.5.1 Early death

49 patients died before remission out of 4732 patients at risk (1% of all patients) (Patients from protocol 58741 are not included in this section because this trial only included patients once in remission).

A progressive significant reduction of deaths before remission has been observed in the last three protocols (58831/2, 58881 and 58951; 2.2%, 0.9% and 0.7% respectively) (Figure 12).

Table 25 – Risk factors during induction in the 58831/2 and 58881 trials

FACTORS	Total	Observed	Expected	*P-value*	Odds ratio	95% Confidence Interval
GENDER				**0.013***	**2.92**	**1.26-6.80**
- Female	1240	21	15.5			
- Male	1559	14	19.5			
AGE (Years)				**0.035**	**1.47**	**1.04-2.10**
- < 1	83	8	1			
- ≥ 1 and < 2	224	3	2.8			
- ≥ 2 and < 6	1447	11	18.1			
- ≥ 6 and < 10	557	4	7			
- ≥ 10	488	9	6.1			
WBC ($x10^9$/l)				0.098	1.43	0.94-2.19
- < 10	1240	16	15.1			
- ≥ 10 and < 25	545	2	6.6			
- ≥ 25 and < 100	639	3	7.8			
- ≥ 100 and < 250	215	5	2.6			
- ≥ 250	157	8	1.9			
NCI Risk Group				**0.001**	**5.25**	**1.99-13.79**
- High	1008	23	12.6			
- Standard	1789	12	22.4			
CNS Involvement				0.059	3.21	0.96-10.77
- Yes	177	4	1.8			
- No	2604	24	26.2			
IMMUNOPHENOTYPE				0.866	0.89	0.24-3.34
- T	393	4	4.9			
- B	2313	29	29.1			
PROTOCOL				**0.000**	**4.69**	**2.10-10.45**
- 58831/2	735	16	9.2			
- 58881	2065	19	25.8			

CNS: Central nervous system; **NCI:** National Cancer Institute**; WBC:** White blood cells
* In grey tone background variables that achieved statistical significance ($p<0.05$)

In the multivariate analysis age, female gender, NCI-High Risk and those having been treated in the 58831/2 protocols were variables significantly associated with an increased risk of dying before remission (Table 25).

Regarding age, patients aged less than one year had an odds ratio of 9.7 (95% CI 4.5-20.7, *p*=0.000) of dying before remission compared with all other groups.

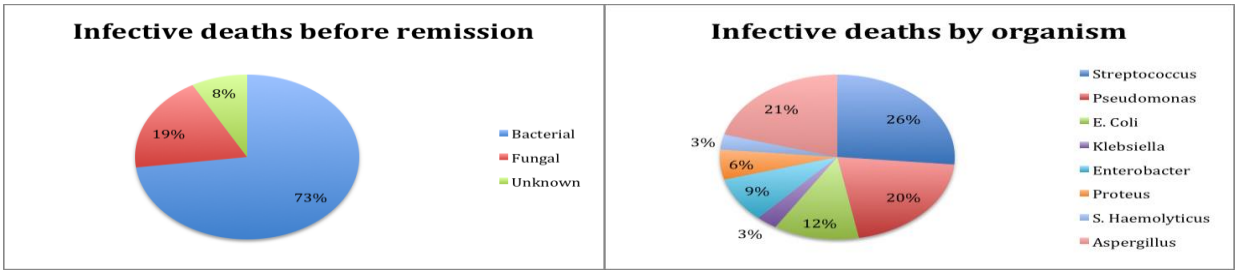
A table summarizing the clinical features and causes of death before remission across all trials is presented below (Table 26).

Before remission, most frequent type of death was infection (n=34, 62.9%). Among infections, bacterial (n=27, 73%) and fungal organisms (n=7, 19%) represented the most frequent causes (Figure 13). Bacterial organisms are mostly represented by *Streptococcus* (n=9, 26%) and *Pseudomonas* (n=7, 21%) while *Aspergillus* was the only fungal infective organism (n=7, 20%) (Figure 14) (Appendix 2, Table A).

Before remission, bleeding disorders were the second most frequent type of death (n=11, 20.4%), mainly intracranial hemorrhages (n=6) and gastrointestinal hemorrhages (n=3). DIC was mostly observed in patients with high WBC at diagnosis and often in the context of an associated infection (n=2). The incidence of tumor burden related deaths has been low across the years when compared with the rest of causes (n=3, 5.6%) (Figure 15) (Appendix 2, Table B). In the last trial, the 58951, non-infective deaths have practically disappeared (n=1; 7.2%).

In the last 58951 trial, there were not patients dying before anti-leukemia treatment could be initiated while there were 7 in the previous trials dying in this period.

One patient dying from necrotizing enterocolitis and pulmonary aspergillosis in trial 58831/2 and 2 patients with ARDS and 1 VOD in the 58881 represent the category of other causes (n=4).



Figures 13-14. Types of infective deaths before remission and by organism

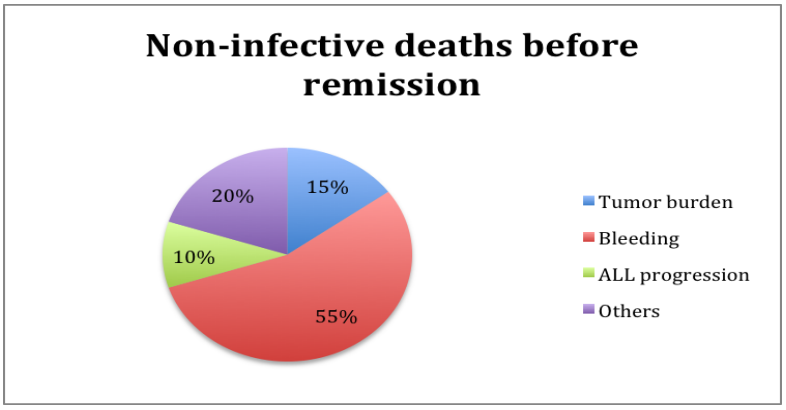


Figure 15. Non-Infective deaths before remission

Table 26- Clinical features and causes of death of all the patients who died before getting into complete remission (Early Death) (%)				
PROTOCOL	**58831/2**	**58881**	**58951**	**TOTAL**
Total	16 (32.7)	19 (38.8)	14 (28.5)	49 (100)
Gender				
▪ **Male**	6 (37.5)	8 (42.1)	5 (35.7)	19 (38.8)
▪ **Female**	10 (62.5)	11 (57.9)	9 (66.3)	30 (61.2)
Time period of death				
▪ **Death before treatment**	2 (12)	5 (26.3)	0 (0)	7 (14.2)
▪ **Death during pre-phase**	0 (0)	5 (26.3)	0 (0)	5 (10.2)
▪ **Death during induction**	14 (88)	9 (47.4)	14 (100)	37 (75.6)
Age groups at diagnosis (Years)				
▪ **< 1**	1 (6.3)	7 (37.1)	0 (0)	8 (16.3)
▪ **1**	1 (6.3)	2 (10.6)	0 (0)	3 (6.1)
▪ **2 -5**	7 (44.1)	4 (21.2)	5 (35.7)	16 (32.6)
▪ **6 - 9**	3 (18.9)	1 (5.3)	5 (35.7)	9 (18.4)
▪ **≥ 10**	4 (25.2)	5 (26.5)	4 (28.5)	13 (26.5)
WBC groups at diagnosis ($x10^9$/l)				
▪ **< 10**	9 (55.8)	7 (37.1)	4 (28.5)	20 (40.8%)
▪ **< 25**	2 (12.6)	0 (0)	4 (28.5)	6 (12.2)
▪ **< 100**	1 (6.3)	2 (10.6)	6 (43)	9 (18.4)
▪ **< 250**	1 (6.3)	4 (21.2)	0 (0)	5 (10.2)
▪ **≥ 250**	3 (18.9)	6 (31.8)	0 (0)	9 (18.4)
ALL immunophenotype				
▪ **B-Lineage**	12 (75.8)	17 (89.4)	10 (75)	39 (79.6)
▪ **T-Lineage**	2 (12.6)	2 (10.6)	4 (25)	8 (16.3)
▪ **Not available**	2 (12.6)	0 (0)	0	2 (4.1)
NCI Risk group				
▪ **Standard**	8 (50)	4 (21.2)	10 (71.4)	22 (44.9)
▪ **High-risk**	8 (50)	15 (78.8)	4 (28.6)	27 (55.1)
Cause of death				
▪ **Tumour burden**	2 (10)	1 (5)	0 (0)	3 (5.6)
▪ **Infection**	11 (55)	10 (50)	13 (92.8)	34 (62.9)
• Bacterial	8	6	13	27
• Viral	0	0	0	0
• Fungal	3	1	3	7
• *Pneumocystis jirovecii*	0	0	0	0
• Unknown	0	3	0	3
▪ **Bleeding/Thrombosis**	5/0 (25)	5/0 (25)	1/0 (7.2)	11/0 (20.4)
▪ **ALL progression**	1 (5)	1 (5)	0 (0)	2 (3.7)
▪ **Others**	1 (5)	3 (15)	0 (0)	4 (7.4)

NCI: National Cancer Institute; **WBC:** White blood cell(s)

1.5.2 Death in first complete remission

123 patients died in complete remission.

A significant reduction of death rates in first complete remission was especially evident between the 58741 protocol (8.1%) and all three others (1.2%, 3% and 1.8% respectively) (Figure 12). On average, the rate of toxic deaths in remission in all four trials was 3.5%. If we exclude the 58741 trial that was conducted early in the 70´s, the rate was 2%.

Table 27 – Risk factors in remission in the 58741 and 58831/2-58881 trials

FACTORS	Total	Observed	Expected	*P-value*	Odds ratio	95% Confidence Interval
GENDER				0.851	0.95	0.56-1.60
- Female	1282	36	38.8			
- Male	1627	52	49.2			
AGE (Years)				**0.022***	**0.709**	**0.53-0.95**
- < 1	72	1	2.2			
- ≥ 1 and < 2	229	7	6.9			
- ≥ 2 and < 6	1512	35	45.7			
- ≥ 6 and < 10	590	16	17.8			
- ≥ 10	506	29	15.3			
WBC (x10^9/l)				0.618	1.068	0.82-1.38
- < 10	1304	27	39.5			
- ≥ 10 and < 25	565	17	17.1			
- ≥ 25 and < 100	669	28	20.2			
- ≥ 100 and < 250	220	11	6.7			
- ≥ 250	150	5	4.5			
NCI Risk Group				0.324	1.43	0.79-2.91
- High	1026	47	31			
- Standard	1882	41	57			
CNS Involvement				0.459	0.65	0.22-2.01
- Yes	173	4	5.2			
- No	2726	83	81.8			
IMMUNOPHENOTYPE				0.698	1.15	0.58-2.28
- T	377	17	10.2			
- B	2313	54	60.8			
HSCT in first remission				**0.000**	**17.07**	**9.02-32.30**
- Yes	92	24	2.8			
- No	2817	64	85.2			
PROTOCOL				**0.000**	**3.22**	**1.81-5.74**
- 58741	184	15	5.6			
- 58831/2-58881	2725	73	82.4			

CNS: Central nervous system; **HSCT:** Hematopoietic stem cell transplantation; **NCI:** National Cancer Institute**;** **WBC:** White blood cell(s)

* In grey tone background variables that achieved statistical significance ($p<0.05$)

In the multivariate analysis age, having been transplanted in first remission and having been treated in the 58741 trial were significantly associated with an increased risk of dying in remission (Table 27).

Regarding age, patients aged >10 years were at a significant increased risk of death in remission when compared with all other groups together (*p*=0.000; OR 2.45; 95% CI 1.51-3.80). Patients above ten years represented 33% of deaths in remission in trials 58741 to 58881.

A table summarizing the clinical features and causes of death in remission across all trials is presented below (Table 28).

Infection was the most frequent cause of death in first CR both on conventional chemotherapy and after HSCT. For those who died of infection receiving conventional chemotherapy in first CR, bacterial infections are the most frequent cause of death (n=18; 36%) (Figure 16). Second and third causes are virus (n=13; 26%) and fungus (n=12; 24%), though the pattern of infective organisms has notably changed (Appendix 2, table A).

In the trial 58741 (1970-1980) four patients deceased due to *Pneumocystis jirovecii* infections. Since the introduction of systematic *Pneumocystis jirovecii* prophylaxis in the early 80´s for protocol 58831/2 and subsequent trials, no related death to this organism has been reported so far until the 58951 trial where one fatal case has been reported. In trial 58741, four patients experienced fatal varicella infections; fatal varicella infections have not been reported during trials 58831/2, 58881 but one case of varicella pneumonia has been described in 58951. On the other hand, in trials 58831/2 and 58881 measles induced pneumopathy has been as the most frequent viral organism (n=4). All these cases happened between 1998 and 1991, 3 were boys and 1 girl. *Aspergillus* remains the most common fungal organism death responsible in first CR (n=8).

Transplant related mortality was high in trials 58831/2 and 58881 (22% and 26% respectively) and became notably lower in the last trial, the 58951 (7%). 31 patients died from HSCT related toxicities (Figure 17). For those who undergo HSCT in first CR, infections represent the most frequent type of death (n=15, 48.8%) (Figure 18). Among infections virus are the most common organisms (40%) followed by fungal infections (33.3%), mainly represented by *Aspergillus*, and in third place are parasites, both cases by *Toxoplasma* (13.3%) (Figure 19). Infective deaths were not reported after HSCT in trial 58831/2, probably due to the global low

number of transplants performed in first CR at that time. Second most frequent cause remains GVHD, followed by hemorrhages, non-specific multiorgan failure (MOF) or related conditioning regimen toxicity (Table 29).

Secondary malignant neoplasms represent an important part of deaths in first CR (11.9%), mainly due to secondary AML and bone/soft tissue sarcomas. By protocol, one patient in the 58741 died of an unspecified brain tumor, eleven patients in the 58881 protocol died of secondary neoplasms (7 AML, one Ewing sarcoma, one adult type sarcoma, one peritoneal carcinomatosis and one pulmonary epithelioma with cerebral metastasis) and 3 in the 58951 (2 AML, 1 medulloblastoma)

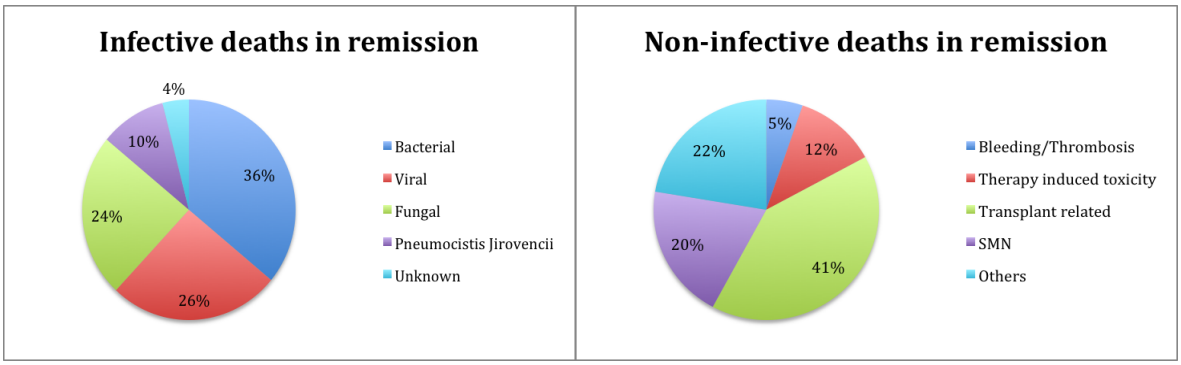
Organ toxicity related toxic deaths are very heterogeneous (n=9, 7.1%). In the 57841 trial three patients died of chemotherapy induced hepatic toxicity. In the 58881 there were two cases of anthracyclines related cardiomyopathy (one of them maybe precipitated by an *Influenza B* infection), one case of toxic epidermal necrolysis (Lyell's syndrome), one acute pancreatitis related to asparaginase, one due to hepatic toxicity that could not be linked to an underlying infection, and one grade IV renal toxicity related to methotrexate and sepsis by *Enterobacter Cloacae.* The local investigators made the assessment of relationship between chemotherapy and death.

Two cases of pulmonary embolism have been reported, one suspected to be of fungal origin and the other one the other one linked to the insertion of a central venous catheter (CVC). One patient in the 58951 trial died of a sepsis of unknown origin, with associated pulmonary hemorrhage and thrombosis.

Seven patients developed a histiocytic disorder and were represented by two cases of malignant systemic histiocytosis in protocol 58831/2, one case of primary HLH and one macrophage activation syndrome (MAS) both in protocol 58881 and three in the 58951, one of which was suspected to be related to a *Flavimonas orzyhabitans* infection.

In two cases, iatrogenia could be considered as the cause of death. First case was a patient who experienced a supraventricular tachycardia while manipulation of the central venous catheter (CVC) during a flushing procedure. Autopsy suggested that death could be related to fat emboli. The second case experienced a pulmonary embolism days after the insertion of a CVC in a child with congenital stenosis of the pulmonary artery. Autopsy did not confirm that this

episode could be related to the cardiac abnormality and the cause of death was assumed to be related to the foreign body.



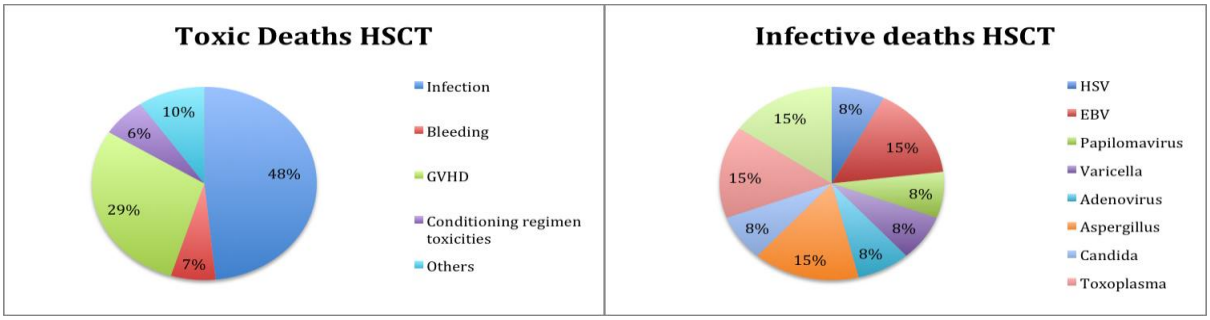
Figures 16 and 17. Infective and non-infective deaths in remission

Table 28- Clinical features and causes of death of all the patients who died in complete remission (%)					
PROTOCOL	**58741**	**58831/2**	**58881**	**58951**	**TOTAL**
Total	15 (12.2)	9 (7.3)	64 (52)	35 (28.5)	123 (100)
Gender					
▪ **Male**	9 (59.8)	7 (77.8)	36 (56.3)	21 (60)	73 (56.9)
▪ **Female**	6 (40.2)	2 (22.2)	28 (33.7)	14 (40)	50 (43.1)
Age groups at diagnosis (Years)					
▪ **< 1**	0 (0)	0 (0)	1 (1.6)	0 (0)	1 (0.8)
▪ **1**	3 (20)	1 (11.1)	3 (4.7)	2 (5.7)	9 (7.3)
▪ **2 -5**	10 (67.6)	6 (66.6)	19 (29.7)	13 (37.1)	48 (39.1)
▪ **6 - 9**	1 (6.7)	0 (0)	15 (23.4)	6 (17.1)	22 (17.9)
▪ **≥ 10**	1 (6.7)	2 (22.2)	26 (40.6)	14 (40)	43 (35)
ALL immunophenotype					
▪ **B-Lineage**	Not available	5 (55.5)	49 (76.6)	27 (77.1)	81 (65.9)
▪ **T-Lineage**	Not available	2 (22.2)	15 (23.4)	8 (22.9)	25 (20.3)
▪ **Unknown/NA**	15 (100)	2 (22.2)	0 (0)	(0)	17 (13.8)
NCI Risk group					
▪ **Standard**	12 (79.9)	6 (66.6)	23 (35.9)	14 (40)	55 (44.7)
▪ **High-risk**	3 (20.1)	3 (33.4)	41 (64.1)	21 (60)	68 (55.3)
Death period					
▪ **Consolidation**	Not comparable	0 (0)	9 (14.1)	5 (14.3)	14 (11.4)
▪ **Interval therapy**	Not comparable	2 (22.2)	3 (4.7)	1 (2.9)	6 (4.9)
▪ **Protocol II**	Not comparable	1 (11.1)	10 (15.6)	7 (20)	18 (14.6)
▪ **Blocs**	Not comparable	0 (0)	2 (3.1)	3 (8.6)	5 (4)
▪ **VANDA**	Not comparable	0 (0)	0 (0)	6 (17.1)	6 (4.8)
▪ **HSCT**	Not comparable	2 (22.2)	22 (34.4)	7 (20)	31 (25.2)
▪ **Maintenance**	Not comparable	4 (44.4)	10 (15.6)	4 (11.4)	18 (14.6)
▪ **Post – treatment**	Not comparable	0 (0)	8 (12.5)	2 (5.7)	10 (8.1)
Cause of death					
▪ **Infection**	11 (73.3)	4 (44.4)	19 (30.9)	16 (44.4)	50 (39.7)
• Bacterial	2	0	8	7	17
• Viral	4	3	4	2	13
• Fungal	0	1	7	4	12
• *Pneumocystis jirovecii*	4	0	0	1	5
• Unknown	0	0	0	2	2
▪ **Bleeding/Thrombosis**	0/0 (0)	0/0 (0)	0/2 (2.9)	1/1 (5.5)	1/3 (3.2)
▪ **Organ toxicity**	3 (20)	0 (0)	6 (8.7)	0 (0)	9 (7.1)
▪ **Transplant related**	0	2 (22.2)	22 (32.3)	7 (19.4)	31 (24.6)
▪ **SMN**	1 (6.7)	0 (0)	11 (16)	3 (8.3)	15 (11.9)
▪ **Others**	0 (0)	3 (33.3)	6 (8.8)	8 (22.2)	17 (13.5)

HSCT: Hematopoietic stem cell transplantation; **NA:** Not available; **NCI:** National Cancer Institute; **SMN:** Secondary malignant neoplasm; **WBC:** White blood cell(s)

Table 29- Causes of death of all the patients who died of transplant related toxicities (%)				
PROTOCOL	**58831/2**	**58881**	**58951**	**TOTAL**
Total	2 (6.4)	22 (71)	7 (22.6)	31 (100)
Cause of death				
▪ **Infection**	0 (0)	11 (50)	4 (57.1)	15 (48.4)
• Bacterial	0	0	0	0
• Viral	0	3	3	6
• Fungal	0	5	0	5
• *Pneumocystis jirovecii*	0	0	0	0
• Parasites	0	2	0	2
• Unknown	0	1	1	2
▪ **Bleeding**	0 (0)	2 (9.1)	0 (0)	2 (6.5)
▪ **GVHD**	0 (0)	7 (31.8)	2 (28.5)	9 (29)
▪ **Conditioning regimen toxicity**	2 (100)	0 (0)	0 (0)	2 (6.5)
▪ **Others**	0 (0)	2 (9.1)	1 (14.4)	3 (9.6)

GVHD: Graft versus host diseases



Figures 18 and 19. Toxic deaths in HSCT and Infective Deaths in HSCT

V. DISCUSSION

1. DISCUSSION

This study provides the largest and most comprehensive report focusing on treatment related deaths in pediatric patients with ALL treated with the EORTC-CLG trials.

The strengths of this study include:

1) The number of patients: 4916 patients from three different countries have been included in this research, representing one of the largest studies on this topic ever performed. This allows a comprehensive analysis.

2) Expanded period of time: since all the EORTC-CLG trials have been included from 1971 to 2008, a wide and detailed perspective about changes in incidence and types of death over this period can be given.

3) Accuracy of the data: data for this study has been obtained from the EORT-CLG databases, a clean and concise deposit of all the information from the different ALL studies. The EORTC-CLG is one of the most reputed research groups in this field worldwide. Some of its investigations have widely contributed to the advancement in pediatric leukemia. A meticulous process of collecting, analyzing and interpreting the data has been put in place for each trial, ensuring the accuracy of the information presented in this study. All deaths have been investigated and 100% of the data regarding the type of death and ultimate cause of decease was available and has been evaluated.

Nevertheless, this study has some limitations:

1) Data from the 59851 trial, the latest one performed by the group from 1998 to 2008, was not mature enough at the time of collecting and analyzing this data. This issue precluded including data from this study in the statistical analysis to identify risk factors for treatment related deaths, one of the objectives of this research. Nonetheless, patients from the 3 other studies (58741, 58831/2, 58881) have been included in this statistical analysis, accounting for 2984 patients. A descriptive analysis of treatment related deaths occurring in the 58951 trial has been presented, permitting to compare the profile of treatment related deaths in the most recent trial with those previous studies.

2) The definition of treatment related mortality: we adopted a definition of treatment related mortality and causes of death used by the BFM, NOPHO and MRC groups[8,43,48]. There is

not a universally established definition in the literature and it may difficult the interpretation and comparison of results.

1.1 Treatment related deaths in pediatric acute lymphoblastic leukemia

Over the last three decades we have witnessed a constant and substantial improvement in the outcome of children and adolescents affected by ALL. Greater than 95% of children with ALL will attain complete remission and around 80% will be long-term survivors[9, 11]. Despite the intensification of antileukemic chemotherapeutic agents, rates of mortality before and after first complete remission have decreased, hence accounting for only a small proportion of failures in the management of children and adolescents with ALL. In view of this increasing overall survival rate, avoidance of treatment-related fatalities and sequelae is a major aim in the optimal management of childhood ALL[10].

In this sense, the EORTC Children's Leukemia Group has applied a risk-adapted therapy background in its protocols, aiming for a more intense treatment in those patients considered at higher risk of relapse, whereas less intensive regimens are given to those in lower risk groups. This is in line with one of the principles of the group that is to reduce the undesirable side effects of the treatment at short and long term and improving the quality of life of these children while maintaining the high rates of cure (www.eortc.org).

1.2 Early death

Despite notable improvements in supportive care at the time of initial diagnosis made during the decade of 1990 and early 2000, there are still a substantial number of patients that die before remission for other reasons than ALL. A progressive reduction in the number of deaths before remission has been observed in our study. In our series, the incidence of deaths before first remission went down from 2.2% in the protocol 58831/2 to 0.7% in the last study, the 58951. This result is similar to those reported by other collaborative groups (Appendix 1). A recent systematic review and meta-analysis of non-relapse mortality (NRM) in acute lymphoblastic leukemia performed over 59 randomized pediatric ALL studies and including 49071 patients revealed an induction death rate of 1.38%[87].

Infection was the most frequent type of death before remission (62.9%). Among infections, bacterial (73%) and fungal organisms (19%) represented the most frequent ones

(Figure 13). In the literature percentages range between 11 and 64% predominately related to bacterial and fungal organisms[7,8,43,47,48] and exceptionally to *Pneumocystis jirovecii*. We did not observe any death before remission related to *Pneumocystis jirovecii.* Bacterial organisms were mostly represented by *Streptococcus* (26%) and *Pseudomonas* (21%) while *Aspergillus* was the only fungal infective organism (20% of all infections).

Besides infection, bleeding disorders were the second most frequent cause of death (20.4%), mainly intracranial (n=6) and gastrointestinal hemorrhages (n=3). Percentage ranges from 0% to 55.5%[7,48] in the literature. Tumor burden related deaths have been low across the three trials (5.6%). In the literature, incidence of tumor burden related deaths range from 0%[8] to 11%[9,47].

We found that patients aged less than one year and those of female gender, NCI-High Risk group patients and those who had been treated in the 58831/2 protocol were at a significantly higher risk of dying before remission (Table 25).

Regarding age we identified that patients aged less than one year had a significant increased risk of dying before remission compared with all other age groups together. Other investigators have also found that younger patients are more prone to die of treatment related complications before remission. Rubnitz et al found that children aged less than 1 and those aged more than 10 years presented a higher risk of death before remission in ALL studies performed in the same period as were those of the EORTC (1984-1999) when compared to patients aged 1 to 9 years[7]. Hargrave et al found that female gender, WBC >50 x 10^9/l and age <2 years were associated with a higher risk in some MRC trials[48].

Regarding gender, we did not find baseline characteristics that could explain the augmented risk of toxic deaths in the female population. Christensen et al also found that girls were significantly at an increased risk of treatment related deaths, particularly in the low-risk groups, B-cell precursor leukemia and in those with less than 100 x 10^9/l WBC[43]. In a recent COG study in children with high risk-ALL, females had significantly more hospital days, delays in therapy, grade 3 or 4 toxicities and supportive care interventions than males. The cumulative incidence of treatment-related deaths was 2.6% for females and 1.2% for males[88]. Maybe the biological mechanism behind these findings may reflect gender differences in immunological response to infections or differences in toxicity following cytotoxic chemotherapy. Gender

differences in response to infection and inflammation have been described in some investigations using animal models as well as human studies[89,90] but has never been implemented in recommendations for clinical management of infections. Few reports have suggested gender differences in mortality rate due to infections following measles vaccinations. One study described a non-specific reduction in mortality following standard measles vaccination, which was particularly associated with female sex[91]. Differences in liver function and related pharmacokinetics could also be contributing factors underlying the observed gender differences in treatment related deaths. While this finding has been suggested in other studies, this research is not able to shed light on the causes of this gender difference.

Regarding NCI Risk, a large proportion among high-risk patients presented with a high-burden of disease (>250 $x10^9$/l WBC, n=8, 35%). Five of them died of intracranial hemorrhages and in two cases DIC was co-responsible of death along with an infection. Therefore, bleeding events and coagulation disorders represent a major concern in this particular population. The high burden of disease in this group of patients may explain its increased risk of death.

Regarding participation to different protocols, it was identified that having been treated in the 58831/2 trial was significantly associated with an increased risk of death before remission when compared with the 58881. Percentages of early death were 2.9% and 0.9% respectively (p=0.000; OR 4.69; 95% CI 2.10-10.45). Pre-phase and induction regimens were superposable in both trials, so treatment differences could not explain by themselves this discrepancy. Types of death were also similar in both trials. When taking into consideration the last 58951 trial, that also used superposable pre-phase and induction regimens, there were not patients dying before anti-leukemia treatment could be initiated nor during the pre-phase, and the early death rate lowered to 0.7%. These two phases are critical, as patients may present in a poor clinical condition and with high-burden of disease.

We therefore hypothesize that this decrease in early deaths can be explained by:

i) The continuous improvement in supportive care measures in the last three decades, such as the implementation of guidelines for management of febrile neutropenia, early use of broad-spectrum antibiotics and the optimization in the use of supportive blood products.

ii) Better recognition of the clinical features making these patients more prone to die so earlier measures can be put in place, such as leukapheresis or earlier admission to intensive care unit. For instance, in our series, more patients with high burden of disease (i.e. WBC >100 $x10^9$) were included in the 58881 trial than in the 58831/2 (14.3% Vs 10.4% respectively, p=0.045), and despite this, the percentage of tumor burden related complications and bleeding lowered (35% Vs 30%).

iii) A progressive learning in treating institutions over the years using similar protocols.

iv) Improved national networks, so that sick patients are referred earlier to reference centers where treatment and supportive care can promptly be initiated.

It is noteworthy that some patients were reported to have more than one cause of death besides infection, particularly bleeding complications, leukostasis and necrotizing enterocolitis. In these cases, infection may had precipitated the fatal event, in an otherwise, fragile patient.

To summarize NCI-High Risk patients, those aged less than one year, female children and those treated in the 58831/2 trial were at an increased risk of death before remission in the EORTC-CLG ALL trials. Patients with these clinical characteristics should be carefully monitored in order to identify earlier signs of toxicity that may prevent them of a fatal outcome.

1.3 Death in first complete remission

In the same systematic review mentioned above, the remission death rate was 1.94% and total non-relapse mortality was 3.60%[87]. In our series, 123 patients died in remission. Most frequent causes of deaths were infection (39.9%), transplant related mortality (26.9%) and secondary malignant neoplasms (13.9%).

The 58741 trial run between 1971 and 1978 and it presented the higher mortality rate of the EORTC trials (8.1%, figure 12). If we exclude this trial, the mortality rate in remission is 2% in average for the three trials. This is in line with what has been reported by other collaborative groups with contemporary trials, where percentage ranges from 1.5%[50] to 5.3%[47].

Reasons behind the differences in treatment related deaths in remission between the 58831/2, 58881 and 58951 trials can be given:

- The number of transplants performed in first remission has progressively increased from the 58831/2 trial to the 58881 and the 58951 (9, 83 and 98 respectively). Despite the increase in

number of transplants, the incidence of transplant related mortality slightly increased between the 58831/2 and the 58881 and notably reduced in the last trial the 58951 (22%, 26% and 7% respectively). As cure rates improved with intensity, transplant related mortality was higher in earlier protocols than in most recent ones. We identified that having been transplanted in first complete remission was significantly associated with an increased risk of death in remission in this period (p=0.000; OR 17.97; 95% CI 9.02-37.3). NCI-High Risk patients were more represented in the transplant group (83.7%) than the standard ones (16.3%), and therefore at an increased risk of developing complications related to this procedure. The difference in transplant related mortality between the last protocol (7.1%) and those two other may be explained by the selection of patients candidates for transplantation, as well as by the better donor selection process and the improvements in the supportive care in the BMT units. Christensen et al also found that being transplanted in first remission was associated with an increased risk of death[43]. In this population, infection remains the most frequent cause of death (48.4%, mainly viral and fungal), followed by GVHD (29%), bleeding (6.5%) and conditioning regimen related toxicity (6.5%).

- Second malignant neoplasms caused 12% (n=15) of deaths in remission in our series and represent the third most frequent type of death in remission. Most frequent type of SMNs related death were AML (n=9, 60%) and solid carcinomas (n=4, 27%). The incidence of SMNs related deaths was higher in the 58881 (n=11, 73% of all SMNs related deaths occurred in this trial). Potential causes for developing SMNs are the use of radiotherapy, etoposide or alkylating agents[92]. In our studies, the value of radiotherapy was tested in a randomized fashion in the 58832 trial, and it was demonstrated that the omission of cranial radiotherapy failed to increase the risk of CNS relapse or of any relapse. This observation led to the deletion of cranial radiotherapy from front-line therapy protocols in subsequent CLCG trials (58881 and 58951)[93]. In our series, only patients treated in the very high-risk group of the 58881 and 58951 trials did receive epipodophyllotoxins in first line (Etoposide cumulative doses 900mg/m^2), but only one patient in the 58881 trial and one in the 58951 were high risk and further developed a secondary neoplasm. Specifically the 58881 protocol looked at the possible oncogenic effect of the high doses of 6-MP given intravenously during continuation therapy in patients assigned to this supplementary treatment by randomization our group compared the two arms, with and without

IV 6-MP, with regard to the occurrence of SMN. No difference could be found between the two groups. For all patients included in the trial (including those with lymphoblastic lymphoma) six patients in the no IV 6-MP arm developed a SMN and five patients in the IV 6-MP group. The type of SMN was similar in the two randomized groups[36].

- Third, deaths related to organ toxicity happened in the 58741 (n=3) and in the 58881 (n=6) and none in the 58831/2 and 58951 trials. Those events happening in the 58741 were all severe hepatic toxicity related to the use of higher doses of MP-6 and MTX than in contemporary trials. In the 58881 protocol, in four cases a clear cause was identified: anthracycline cardiomyopathy induced (n=2), asparaginase toxicity (n=1) and methotrexate induced renal toxicity (n=1). Contemporary ALL trials included clear guidelines on cardiac toxicity monitoring, methotrexate toxicity management and asparaginase modifications in case of allergic reactions or organ toxicity. Besides this, an increased awareness and early recognition of therapy-induced toxicity has probably led to the reduction of deaths in this category.

The incidence of infective deaths has decreased from the 58741 (n=11 [73%]) to the 58831/2, 58881 and 58951 (n=4 [44%], n=22 [30.9%], n=16 [44.4%]) and the pattern of infections has changed. Notably, the number of cases of *P. Jiroveciii* has reduced from 4 cases in the 58741 to none in the 58831/2 and 58881 and only one in the 58951. This improvement is surely related to the introduction of specific prophylaxis in the 80's[94]. Besides this, patients in the 58741 were treated with high doses of steroids (up to 120mg/m^2 of prednisone during 28 days of induction) and non-conventional doses of Asparaginase (up to 150.000 IU/m^2), which is significantly higher than the doses currently used in our contemporary trials. Varicella was a common cause of death in the 58741 trial while measles infection occurred in the 58831/2 and 58881 trials. *Aspergillus* is the most common cause of fungal infection across all trials. Widespread MMR vaccination in the decades of the 80´s and 90´s has contributed to the reduction of these infections in immune-competent individuals and the risk of transmission to immune-deficient patients, therefore reducing the risk of dying from this complication, particularly in the ALL population (www.vacunasaep.org).

Regarding risk factors, we identified that patients above 10 years were at an increased risk for death in remission. Adolescent are known high-risk subgroup for treatment related deaths in ALL. Hunger et al found that patients aged 10+ years had a relative risk of death up to 3 times

fold compared to those aged 1 to 9.99 years in two time periods (1990-1994) and (2000-2005)[95]. Treatment related toxicities are more common in patients aged more than 10 years, particularly pancreatitis and thromboembolic events[96]. Adolescents do experience more toxic events when are treated in pediatric protocols than when they are treated with adult regimens due to the use of more intensified regimens[97]. Underlying biological differences and a different tolerance to the same chemotherapeutic agents, may make older patients more prone to experience fatal events.

The 57841 trial was significantly associated with a higher risk of death in remission when compared with the two other protocols (p=0.000; OR 3.22; 95% CI 1.81-5.74). The therapeutic approach between the 58741 and the others radically change since the introduction of a BFM based schema from the 58831/2 trials and onwards. The 58741 trial has the highest incidence of infective deaths: 73.3% Vs 38.2 in the 58831/2-58881 trials. This trial was performed early in the decade of 1970, and neither supportive care measures nor adequate anti-microbial therapies were as we know nowadays. These differences may explain the high rate of mortality in that first trial.

Death in remission remains an important issue in pediatric cancer. A cohort of 6402 Italian patients with cancer aged less than 18 years at diagnosis were followed up after remission[98]. 890 died, mostly due to relapse (84.6%). Most frequent causes of death other than first cancer were second cancers (6.7%), infection (2.1%) and cardiac diseases (1.3%). Subjects after leukemia and lymphoma were more likely to die due to medical complications of therapy compared to patients with solid tumors. Second cancers were the second most frequent cause of death, with a 12-fold risk compared with the general population. Compared with the general population, these subjects were 32 times more likely than same-age subjects to die.

To summarize patients above 10 years, those transplanted in first remission and those treated in the 58741 trial were at an increased risk of death in remission in the EORTC-CLG ALL trials.

1.4 Definition of treatment related mortality

We opted for a definition of treatment related mortality and the types and causes of death like the BFM, NOPHO or MRC groups have described it[8,43,48] but this definition is not homogenously used in the scientific community. Chantal MC and co-workers conducted a systematic review of randomized therapeutic pediatric acute leukemia and adult/pediatric acute promyelocytic leukemia trials[99]. Only 6% of the pediatric studies clearly defined treatment

related mortality in the material section; of note, in pediatric studies specifically focused on treatment related mortality, only 70% of them clearly defined this concept. Between 18 and 30% of the studies include deaths after completing chemotherapy in the definition of treatment related mortality and between 18 and 70% included deaths after stem cell transplant into it. They also raised the question of inconsistency in defining attribution for cause of death. First because a reliable system of attribution related to treatment related mortality has never been developed and second, because patients will have multiple events close to death, such as organ dysfunction, infection, and hemorrhage and currently, there are no clear ways to classify the primary cause of death for these patients.

In order to shed light into this issue, a recent systematic assessment from the International Pediatric Oncology Mortality Classification Group, has developed a definition of treatment related mortality in pediatric cancer patients[100]. Treatment-related mortality is defined in this paper as any death in the absence of progressive cancer. They also develop a cause of death attribution system and the clinical criteria for assigning probable cause-of-death and possible cause-of-death for each category. Such initiatives are necessary in order to homogenize the way in which physicians define treatment related mortality that will permit to compare results from different treatment regimens in pediatric patients.

VI. CONCLUSIONS

1. CONCLUSIONS

Objective: To assess the types and causes of treatment related deaths in children with newly diagnosed ALL treated according to the first line EORTC-CLG ALL protocols 58741 – 58831/2, 58881 and 58951 from 1971 to 2008.

Conclusion: In this study we have described the incidence and type of treatment related deaths in children with newly diagnosed ALL treated in the EORT-CLG trials. The analysis of 4916 patients has permitted to identify factors for increased likelihood of dying from toxicity. The results have been compared with those reported in the literature from collaborative groups.

Objective: To define the incidence and causes of treatment related deaths in the four trials grouped in two categories: early death (death before first complete remission) and death in first complete remission.

Conclusion:

- Incidence of early deaths in our series is 1% on average across trials 58831/2, 58881 and 58951. Most frequent types of death before remission are: 1) Infection: 62.9%, of which bacterial are 73% and fungal 19%; 2) Bleeding: 20.4%; 3) Tumor burden: 5.6%.
- Incidence of death in first complete remission in our series is 3.5%. If we exclude the 58741 trial that has the highest incidence of toxic deaths (8.1%) and was conducted early in the decade of the 70´s, the incidence lowers to 2%. Most frequent types of death in remission are: 1) Infection: 39.7%, of which bacterial are 36%, viral 26% and fungal 24%; 2) Bone marrow transplant related complications: 24.6%; 3) Secondary malignant neoplasms: 11.4%; 4) Organ toxicity: 7.1%.

Objective: To assess whether the treatment era carries an impact in the incidence of treatment related deaths.

Conclusion:

- Incidence of all treatment related deaths have reduced from the 58741 protocol (8.1%) performed during the decade of the 70´s to the three other trials 58831/2, 58881 and 58951 (3.4%, 3.9% and 2.5% respectively) performed in the decades of the 80´s, 90´s and 2000.
- Incidence of early deaths progressively reduced between the 58831/2, 58881 and the 58951 trials (2.2%, 0.9% and 0.7% respectively).

- Incidence of deaths in first complete remission reduced from the 8.1% in the 58741 trial to 1.2% in the 58831/2, 3% in the 58881 and 1.8% in the last trial, the 58951.
- The reduction in all treatment related deaths, deaths before remission and deaths in remission is statistically significant (p<0.001).
- The multivariate analysis identified that:
    - Before remission, having been treated in the 588831/2 conferred a significant increase in the risk of dying from toxicity when compared to the 58881 trial (p=0.000; Odds ratio 4.69; 95% CI 2.10-10.45)
    - In first complete remission, having been treated in the 58741 trial conferred a significant increase in the risk of dying from toxicity when compared to the other two trials, 58831/2 and 58881 (p=0.000; Odds ratio 3.22; 95% CI 1.81-3.74).
- Therefore, the time period in which patients were treated impacts in the risk of dying from toxicity in the EORTC-CLG trials.

Objective: To identify risk factors of increased likelihood of treatment related deaths in children with ALL.

Conclusion:

- The multivariate analysis identified that:
    - Before remission, patients aged less than one year (p=0.000, OR 9.7; 95% CI 4.5-20.7), those of female gender (p=0.013; OR 2.92; 95% CI 1.26-6.80) and those NCI-High Risk (p=0.001; OR 5.25; 95% CI 1.99-13.79) were at a significantly increased risk of dying before remission
    - In first complete remission, patients aged more than 10 years (p=0.000; OR 2.45; 95% CI 1.51-3.80) and those having been transplanted in first remission (p=0.000; OR 17.07; 95% CI 9.02-32.30) were at a significantly increased risk of death in remission.

Objective: To compare the incidence and type of treatment related deaths of the EORTC-CLG ALL protocols with the results reported by other collaborative groups.

Conclusion:

- Publications from collaborative study groups focusing on treatment related mortality in ALL pediatric patients treated in front line protocols were retrieved and analyzed.

- Incidence of early deaths in our series is 1% on average across trials 58831/2, 58881 and 58951. This is lower compared with what has been reported by other collaborative groups with contemporary trials, where percentage is 1.4%. Most frequent causes are infection and bleeding disorders in our series; these are the most frequent causes reported by other groups.
- Incidence of death in complete remission in our series is 3.5% on average across trials 58741, 58831/2, 58881 and 58951. If we exclude the 58741 trial that has the highest incidence of toxicity and was performed early in the decade of the 70´s, the incidence lowers to 2%. This is similar compared with what has been reported by other collaborative groups with contemporary trials, where percentage is 1.94%. Most frequent causes are infection, toxicities derived from transplantation and secondary malignant neoplasms in our series; besides these causes, drug-induced toxicity has been a frequent cause reported by other groups.

2. CONCLUSIONES

Objetivo: Describir los tipos y causas de mortalidad relacionada con el tratamiento en niños con leucemia linfoblástica aguda de nuevo diagnóstico tratados en los protocolos de primera línea del grupo EORTC-CLG 58741, 58831/2, 58881 y 58951 desde 1971 a 2008.

Conclusión:

- En este estudio hemos descrito la incidencia y el tipo de muertes relacionadas con el tratamiento en niños de nuevo diagnóstico de leucemia linfoblástica (LLA) aguda tratados con los protocolos EORTC-CLG. El análisis de 4916 pacientes ha permitido identificar factores de riesgo para fallecer por causas tóxicas. Estos resultados han sido comparados con los publicados por otros grupos colaborativos.

Objetivo: Definir la incidencia y causas de muerte relacionadas con el tratamiento en los cuatro ensayos clínicos agrupados en dos categorías: Muerte precoz o muerte antes de la primera remisión completa y Muerte en primera remisión completa.

Conclusión:

- La incidencia de muerte precoz en esta serie es del 1% de media entre los protocolos 58831/2, 58881 y 58951. Las causas más frecuentes de muerte precoz son: 1) Infección: 62,9%, de las cuales el 73% es bacteriana y el 19% fúngica; 2) Hemorragia: 20,4%; 3) Carga tumoral: 5,6%.
- La incidencia de muerte en primera remisión completa en esta serie es 3,5%. Si excluimos el protocolo 58741 que tiene la mayor incidencia de muerte tóxica (8,1%) y se llevó a cabo en la década de los 70, la incidencia baja al 2%. Las causas más frecuentes de muerte en remisión son: 1) Infección: 39,7%, de las cuales el 36% es bacteriana, el 26% viral y el 24% fúngica; 2) Toxicidad relacionada con el trasplante: 24,6%; 3) Segundas neoplasias: 11,4%; 4) Toxicidad orgánica: 7,1%.

Objetivo: Averiguar si la era de tratamiento impacta en la incidencia de muertes relacionadas con el tratamiento.

Conclusión:

- La incidencia de todas las muertes relacionadas con el tratamiento se ha reducido desde el protocolo 58741 (8,1%) llevado a cabo en la década de los 70 a los otros tres protocolos, el 58831/2, 58881 y 58951 (3,4%, 3,9% y 2,5% respectivamente) llevados a cabo en las décadas de los 80, 90 y 2000 respectivamente.

- La incidencia de mortalidad precoces se ha reducido progresivamente entre los protocolos 58831/2, 58881 y 58951 (2,2%, 0,9% y 0,7% respectivamente).
- La incidencia de mortalidad en primera remisión se ha reducido desde el 8,1% del protocolo 58741 al 1,2% en el 58831/2, 3% en el 58881 y 1,8% en el último protocolo, el 58951.
- La reducción en la incidencia de todas las muertes relacionadas con el tratamiento, las muertes precoces y en primera remisión es estadísticamente significativa (p<0,001).
- El análisis multi-variante ha identificado que:
    - Previo a la remisión, el haber sido tratado en el protocolo 58831/2 confirió un mayor riesgo de mortalidad tóxica cuando se comparó con el 58881 (p=0,000; Odds ratio 4,69; IC 95% 2,10-10,45).
    - En primera remisión, el haber sido tratado en el protocolo 58741 confirió un mayor riesgo de mortalidad tóxica cuando se comparó con los otros dos protocolos, el 58831/2 y el 58881 (p=0,000; Odds ratio 3,22%; IC 95% 1,81-3,74).
    - Por tanto, el periodo de tiempo en el que los pacientes se trataron impacta en el riesgo de mortalidad relacionada con el tratamiento en los protocolos EORTC-CLG.

Objetivo: Identificar factores de riesgo para fallecer de muerte tóxica en pacientes pediátricos con LLA.

Conclusión:

- El análisis multi-variante ha identificado que:
    - Antes de la remisión, los pacientes menores de un año (p=0,000, OR 9,7; IC 95% 4,5-20,7), aquellos de sexo femenino (p=0,013; OR 2,92; IC 95% CI 1,26-6,80) y aquellos del grupo de alto riesgo NCI (p=0,001; OR 5,25; IC 95% 1,99-13,79) tenían un riesgo significativamente más alto de experimentar mortalidad relacionada con el tratamiento antes de la remisión.
    - En primera remisión, los pacientes de más de 10 años (p=0,000; OR 2,45; IC 95% 1,51-3,80) y aquellos trasplantados en primera remisión (p=0.000; OR 17.07; 95% CI 9.02-32.30) tenían un riesgo significativamente más alto de experimentar mortalidad relacionada con el tratamiento en primera remisión.

Objetivo: Comparar la incidencia, el tipo y la causa de mortalidad relacionada con el tratamiento de los protocolos EORTC-CLG con los resultados publicados por otros grupos colaborativos.

Conclusión:

- Se identificaron y analizaron publicaciones de grupos colaborativos centradas en el la mortalidad relacionada con el tratamiento en protocolos de primera línea en LLA.
- La incidencia en nuestra serie de mortalidad precoz es del 1% de media entre los protocolos 58831/2, 58881 y 58951. Esta incidencia es menor cuando se compara con la descrita por otros grupos con estudios contemporáneos, en el que el porcentaje es del 1,4%. En esta serie las causas más frecuentes son infección y sangrado que coincide con las causas más frecuentes descritas en la literatura.
- La incidencia en nuestra serie de mortalidad en primera remisión es del 3,5% de media entre los protocolos 58741, 58831/2, 58881 y 58951. Si excluimos el protocolo 58741 que tiene la más alta incidencia de mortalidad tóxica y que se llevó a cabo en la década de los 70, la incidencia baja al 2%. Esta incidencia es similar a la descrita por otros grupos, que está alrededor del 1,94%. En esta serie las causas más frecuentes son infección, toxicidades derivadas del trasplante y segundas neoplasias. Además de estas tres, en la literatura la toxicidad orgánica ha sido una causa frecuente descrita por otros grupos.

VII. REFERENCES

1. LIST OF REFERENCES

1. Boyle P, Levin B, editors. World cancer report in 2008. In: World cancer report in 2008. Lyon: International Agency for Research on Cancer; 2008. p. 482–7.
2. Steliarova-Foucher E, Stiller C, Lacour B, Kaatsch P. International Classification of Childhood Cancer, third edition. Cancer 2005;103(7):1457–67.
3. Imbach P, Khune T, Arceci J. Pediatric oncology: A comprehensive guide. In: Imbach P, Khune T, Arceci J, editors. Pediatric oncology: A comprehensive guide. Berlin: Springer; 2011. p. XVII – XIX.
4. Silverman L, Sallan S. Hematology of infancy and childhood. In: Nathan D, Oski F, editors. Hematology of infancy and childhood. Philadelphia: Saunders; 2003. p. 1135–66.
5. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976;33(4):451–8.
6. Pui C-H, Yang JJ, Hunger SP, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol 2015;33(27):2938–48.
7. Rubnitz JE, Lensing S, Zhou Y, et al. Death during induction therapy and first remission of acute leukemia in childhood: the St. Jude experience. Cancer 2004;101(7):1677–84.
8. Prucker C, Attarbaschi A, Peters C, et al. Induction death and treatment-related mortality in first remission of children with acute lymphoblastic leukemia: a population-based analysis of the Austrian Berlin-Frankfurt-Münster study group. Leukemia 2009;23(7):1264–9.
9. Slats AM, Egeler RM, van der Does-van den Berg A, et al. Causes of death--other than progressive leukemia--in childhood acute lymphoblastic (ALL) and myeloid leukemia (AML): the Dutch Childhood Oncology Group experience. Leukemia 2005;19(4):537–44.
10. Pui C-H, Relling M V, Downing JR. Acute lymphoblastic leukemia. N Engl J Med 2004;350(15):1535–48.
11. Beneficial and harmful effects of anthracyclines in the treatment of childhood acute lymphoblastic leukaemia: a systematic review and meta-analysis. Br J Haematol 2009;145(3):376–88.
12. Eden TOB, Pieters R, Richards S. Systematic review of the addition of vincristine plus steroid pulses in maintenance treatment for childhood acute lymphoblastic leukaemia - an individual patient data meta-analysis involving 5,659 children. Br J Haematol 2010;149(5):722–33.
13. Escherich G, Richards S, Stork LC, Vora AJ. Meta-analysis of randomised trials comparing thiopurines in childhood acute lymphoblastic leukaemia. Leukemia 2011;25(6):953–9.
14. Richards S, Pui C-H, Gayon P. Systematic review and meta-analysis of randomized trials of central nervous system directed therapy for childhood acute lymphoblastic leukemia. Pediatr Blood Cancer 2013;60(2):185–95.
15. Pui C-H, Schrappe M, Masera G, et al. Ponte di Legno Working Group: statement on the right of children with leukemia to have full access to essential treatment and report on the Sixth International Childhood Acute Lymphoblastic Leukemia Workshop. Leukemia 2004;18(6):1043–53.
16. Hunger SP, Baruchel A, Biondi A, et al. The thirteenth international childhood acute

lymphoblastic leukemia workshop report: La Jolla, CA, USA, December 7-9, 2011. Pediatr Blood Cancer 2013;60(2):344–8.
17. Pui C-H, Chessells JM, Camitta B, et al. Clinical heterogeneity in childhood acute lymphoblastic leukemia with 11q23 rearrangements. Leukemia 2003;17(4):700–6.
18. Pui C-H, Gaynon PS, Boyett JM, et al. Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet (London, England) 2002;359(9321):1909–15.
19. Nachman JB, Heerema NA, Sather H, et al. Outcome of treatment in children with hypodiploid acute lymphoblastic leukemia. Blood 2007;110(4):1112–5.
20. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet 2013;45(3):242–52.
21. Aricò M, Valsecchi MG, Camitta B, et al. Outcome of treatment in children with Philadelphia chromosome-positive acute lymphoblastic leukemia. N Engl J Med 2000;342(14):998–1006.
22. Aricò M, Schrappe M, Hunger SP, et al. Clinical outcome of children with newly diagnosed Philadelphia chromosome-positive acute lymphoblastic leukemia treated between 1995 and 2005. J Clin Oncol 2010;28(31):4755–61.
23. Biondi A, Schrappe M, De Lorenzo P, et al. Imatinib after induction for treatment of children and adolescents with Philadelphia-chromosome-positive acute lymphoblastic leukaemia (EsPhALL): a randomised, open-label, intergroup study. Lancet Oncol 2012;13(9):936–45.
24. Mullighan CG, Su X, Zhang J, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med 2009;360(5):470–80.
25. Den Boer ML, van Slegtenhorst M, De Menezes RX, et al. A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol 2009;10(2):125–34.
26. Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014;371(11):1005–15.
27. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol 2009;10(2):147–56.
28. Buitenkamp TD, Izraeli S, Zimmermann M, et al. Acute lymphoblastic leukemia in children with Down syndrome: a retrospective analysis from the Ponte di Legno study group. Blood 2013;123(1):70–7.
29. Schrappe M, Hunger SP, Pui C-H, et al. Outcomes after induction failure in childhood acute lymphoblastic leukemia. N Engl J Med 2012;366(15):1371–81.
30. Pinkel D. Chilhood leukemias. In: Pui C, editor. Chilhood leukemias. Cambridge: Cambridge University Press; 2006. p. 3–20.
31. Styczynski J, Gil L. Prevention of infectious complications in pediatric HSCT. Bone Marrow Transplant 2008;42 Suppl 2:S77–81.
32. Verdeguer A, de Heredia CD, González M, et al. Observational prospective study of viral infections in children undergoing allogeneic hematopoietic cell transplantation: a 3-year GETMON experience. Bone Marrow Transplant 2011;46(1):119–24.
33. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med 2010;363(22):2091–101.

34. Pui CH, Behm FG, Raimondi SC, et al. Secondary acute myeloid leukemia in children treated for acute lymphoid leukemia. N Engl J Med 1989;321(3):136–42.
35. Nottage K, Lanctot J, Li Z, et al. Long-term risk for subsequent leukemia after treatment for childhood cancer: a report from the Childhood Cancer Survivor Study. Blood 2011;117(23):6315–8.
36. Renard M, Suciu S, Bertrand Y, et al. Second neoplasm in children treated in EORTC 58881 trial for acute lymphoblastic malignancies: low incidence of CNS tumours. Pediatr Blood Cancer 2011;57(1):119–25.
37. Castagnola E, Fratino G, Valera M, Giacchino M, Haupt R, Molinari AC. Correlation between "malfunctioning events" and catheter-related infections in pediatric cancer patients bearing tunneled indwelling central venous catheter: results of a prospective observational study. Support Care Cancer 2005;13(9):757–9.
38. Fratino G, Molinari AC, Parodi S, et al. Central venous catheter-related complications in children with oncological/hematological diseases: an observational study of 418 devices. Ann Oncol 2005;16(4):648–54.
39. Bautista F, Gómez-Chacón J, Costa E, et al. Retained intravascular fragments after removal of indwelling central venous catheters: a single institution experience. J Pediatr Surg 2010;45(7):1491–5.
40. Seger AC. Inadvertent intrathecal administration of intravenous vincristine. J Pediatr Hematol Oncol 2010;32(2):167.
41. Womer RB, Tracy E, Soo-Hoo W, Bickert B, DiTaranto S, Barnsteiner JH. Multidisciplinary systems approach to chemotherapy safety: rebuilding processes and holding the gains. J Clin Oncol 2002;20(24):4705–12.
42. Lund B, Åsberg A, Heyman M, et al. Risk factors for treatment related mortality in childhood acute lymphoblastic leukaemia. Pediatr Blood Cancer 2011;56(4):551–9.
43. Christensen MS, Heyman M, Möttönen M, Zeller B, Jonmundsson G, Hasle H. Treatment-related death in childhood acute lymphoblastic leukaemia in the Nordic countries: 1992-2001. Br J Haematol 2005;131(1):50–8.
44. Vilmer E, Suciu S, Ferster A, et al. Long-term results of three randomized trials (58831, 58832, 58881) in childhood acute lymphoblastic leukemia: a CLCG-EORTC report. Children Leukemia Cooperative Group. Leukemia 2000;14(12):2257–66.
45. Pui C-H, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med 2009;360(26):2730–41.
46. Balduzzi A, Valsecchi MG, Silvestri D, et al. Transplant-related toxicity and mortality: an AIEOP prospective study in 636 pediatric patients transplanted for acute leukemia. Bone Marrow Transplant 2002;29(2):93–100.
47. Atra A, Richards SM, Chessells JM. Remission death in acute lymphoblastic leukaemia: a changing pattern. Arch Dis Child 1993;69(5):550–4.
48. Hargrave DR, Hann II, Richards SM, et al. Progressive reduction in treatment-related deaths in Medical Research Council childhood lymphoblastic leukaemia trials from 1980 to 1997 (UKALL VIII, X and XI). Br J Haematol 2001;112(2):293–9.
49. Mitchell C, Richards S, Harrison CJ, Eden T. Long-term follow-up of the United Kingdom medical research council protocols for childhood acute lymphoblastic leukaemia, 1980-2001. Leukemia 2010;24(2):406–18.
50. Tsurusawa M, Shimomura Y, Asami K, et al. Long-term results of the Japanese

Childhood Cancer and Leukemia Study Group studies 811, 841, 874 and 911 on childhood acute lymphoblastic leukemia. Leukemia 2010;24(2):335–44.
51. Tsuchida M, Ikuta K, Hanada R, et al. Long-term follow-up of childhood acute lymphoblastic leukemia in Tokyo Children's Cancer Study Group 1981-1995. Leukemia 2000;14(12):2295–306.
52. Silverman LB, Stevenson KE, O'Brien JE, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia 2010;24(2):320–34.
53. Schrappe M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Münster. Leukemia 2000;14(12):2205–22.
54. Harms DO, Janka-Schaub GE. Co-operative study group for childhood acute lymphoblastic leukemia (COALL): long-term follow-up of trials 82, 85, 89 and 92. Leukemia 2000;14(12):2234–9.
55. Escherich G, Horstmann MA, Zimmermann M, Janka-Schaub GE. Cooperative study group for childhood acute lymphoblastic leukaemia (COALL): long-term results of trials 82,85,89,92 and 97. Leukemia 2010;24(2):298–308.
56. Conter V, Aricò M, Valsecchi MG, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) acute lymphoblastic leukemia studies, 1982-1995. Leukemia 2000;14(12):2196–204.
57. Conter V, Aricò M, Basso G, et al. Long-term results of the Italian Association of Pediatric Hematology and Oncology (AIEOP) Studies 82, 87, 88, 91 and 95 for childhood acute lymphoblastic leukemia. Leukemia 2010;24(2):255–64.
58. Gaynon PS, Angiolillo AL, Carroll WL, et al. Long-term results of the children's cancer group studies for childhood acute lymphoblastic leukemia 1983-2002: a Children's Oncology Group Report. Leukemia 2010;24(2):285–97.
59. Kamps WA, van der Pal-de Bruin KM, Veerman AJP, Fiocco M, Bierings M, Pieters R. Long-term results of Dutch Childhood Oncology Group studies for children with acute lymphoblastic leukemia from 1984 to 2004. Leukemia 2010;24(2):309–19.
60. Salzer WL, Devidas M, Carroll WL, et al. Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984-2001: a report from the children's oncology group. Leukemia 2010;24(2):355–70.
61. Stark B, Nirel R, Avrahami G, et al. Long-term results of the Israeli National Studies in childhood acute lymphoblastic leukemia: INS 84, 89 and 98. Leukemia 2010;24(2):419–24.
62. Stary J, Jabali Y, Trka J, et al. Long-term results of treatment of childhood acute lymphoblastic leukemia in the Czech Republic. Leukemia 2010;24(2):425–8.
63. Liang D-C, Yang C-P, Lin D-T, et al. Long-term results of Taiwan Pediatric Oncology Group studies 1997 and 2002 for childhood acute lymphoblastic leukemia. Leukemia 2010;24(2):397–405.
64. Liang DC, Hung IJ, Yang CP, et al. Unexpected mortality from the use of E. coli L-asparaginase during remission induction therapy for childhood acute lymphoblastic leukemia: a report from the Taiwan Pediatric Oncology Group. Leukemia 1999;13(2):155–60.
65. Silverman LB, Declerck L, Gelber RD, et al. Results of Dana-Farber Cancer Institute

Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1981-1995). Leukemia 2000;14(12):2247–56.
66. Laosombat V, Wongchanchailert M, Sattayasevana B, Wiriyasateinkul A, Watana-Areporncha i S. The treatment of children with acute lymphoblastic leukemia in Thailand. Med Pediatr Oncol 2002;38(4):266–8.
67. Howard SC, Pedrosa M, Lins M, et al. Establishment of a pediatric oncology program and outcomes of childhood acute lymphoblastic leukemia in a resource-poor area. JAMA 2004;291(20):2471–5.
68. Metzger ML, Howard SC, Fu LC, et al. Outcome of childhood acute lymphoblastic leukaemia in resource-poor countries. Lancet 2003;362(9385):706–8.
69. Advani S, Pai S, Venzon D, et al. Acute lymphoblastic leukemia in India: an analysis of prognostic factors using a single treatment regimen. Ann Oncol 1999;10(2):167–76.
70. Gupta S, Bonilla M, Fuentes SL, et al. Incidence and predictors of treatment-related mortality in paediatric acute leukaemia in El Salvador. Br J Cancer 2009;100(7):1026–31.
71. Belgaumi AF, Al-Bakrah M, Al-Mahr M, et al. Dexamethasone-associated toxicity during induction chemotherapy for childhood acute lymphoblastic leukemia is augmented by concurrent use of daunomycin. Cancer 2003;97(11):2898–903.
72. Stryckmans PA, Otten J, Delbeke MJ, et al. Comparison of chemotherapy with immunotherapy for maintenance of acute lymphoblastic leukemia in children and adults. Blood 1983;62(3):606–15.
73. Cavé H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer--Childhood Leukemia Cooperative Group. N Engl J Med 1998;339(9):591–8.
74. Clappier E, Collette S, Grardel N, et al. NOTCH1 and FBXW7 mutations have a favorable impact on early response to treatment, but not on outcome, in children with T-cell acute lymphoblastic leukemia (T-ALL) treated on EORTC trials 58881 and 58951. Leukemia 2010;24(12):2023–31.
75. Domenech C, Suciu S, De Moerloose B, et al. Dexamethasone (6 mg/m2/day) and prednisolone (60 mg/m2/day) were equally effective as induction therapy for childhood acute lymphoblastic leukemia in the EORTC CLG 58951 randomized trial. Haematologica 2014;99(7):1220–7.
76. Clappier E, Grardel N, Bakkus M, et al. IKZF1 deletion is an independent prognostic marker in childhood B-cell precursor acute lymphoblastic leukemia, and distinguishes patients benefiting from pulses during maintenance therapy: results of the EORTC Children's Leukemia Group study 58951. Leukemia 2015;29(11):2154–61.
77. Favara BE, Feller AC, Pauli M, et al. Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society. Med Pediatr Oncol 1997;29(3):157–66.
78. Smith M, Arthur D, Camitta B, et al. Uniform approach to risk classification and treatment assignment for children with acute lymphoblastic leukemia. J Clin Oncol 1996;14(1):18–24.
79. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84(5):1361–92.

80. Trinkle R, Wu JK. Errors involving pediatric patients receiving chemotherapy: a literature review. Med Pediatr Oncol 1996;26(5):344–51.
81. Aur RJ, Hustu HO, Verzosa MS, Wood A, Simone J V. Comparison of two methods of preventing central nervous system leukemia. Blood 1973;42(3):349–57.
82. Langermann HJ, Henze G, Wulf M, Riehm H. [Estimation of tumor cell mass in childhood acute lymphoblastic leukemia: prognostic significance and practical application]. Klin Pädiatrie 194(4):209–13.
83. Wright DH. Updated Kiel classification for lymphomas. J Pathol 1989;157(4):283–4.
84. Duval M, Suciu S, Ferster A, et al. Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer-Children's Leukemia Group phase 3 trial. Blood 2002;99(8):2734–9.
85. Mahmoud HH, Rivera GK, Hancock ML, et al. Low leukocyte counts with blast cells in cerebrospinal fluid of children with newly diagnosed acute lymphoblastic leukemia. N Engl J Med 1993;329(5):314–9.
86. Henze G, Buchmann S, Fengler R, Hartmann R. The BFM relapse studies in childhood ALL: concepts of two multicenter trials and results after 2 1/2 years. Haematol Blood Transfus 1987;30:147–55.
87. Blanco E, Beyene J, Maloney AM, et al. Non-relapse mortality in pediatric acute lymphoblastic leukemia: a systematic review and meta-analysis. Leuk Lymphoma 2012;53(5):878–85.
88. Meeske KA, Ji L, Freyer DR, et al. Comparative Toxicity by Sex Among Children Treated for Acute Lymphoblastic Leukemia: A Report From the Children's Oncology Group. Pediatr Blood Cancer 2015;62(12):2140–9.
89. Curiel RE, Miller MH, Ishikawa R, Thomas DC, Bigley NJ. Does the gender difference in interferon production seen in picornavirus-infected spleen cell cultures from ICR Swiss mice have any in vivo significance? J Interferon Res 1993;13(6):387–95.
90. Spitzer JA, Zhang P. Gender differences in phagocytic responses in the blood and liver, and the generation of cytokine-induced neutrophil chemoattractant in the liver of acutely ethanol-intoxicated rats. Alcohol Clin Exp Res 1996;20(5):914–20.
91. Aaby P, Jensen H, Samb B, et al. Differences in female-male mortality after high-titre measles vaccine and association with subsequent vaccination with diphtheria-tetanus-pertussis and inactivated poliovirus: reanalysis of West African studies. Lancet (London, England) 2003;361(9376):2183–8.
92. Hawkins MM, Wilson LM, Stovall MA, et al. Epipodophyllotoxins, alkylating agents, and radiation and risk of secondary leukaemia after childhood cancer. BMJ 1992;304(6832):951–8.
93. Otten J, Philippe N, Suciu S, et al. The Children Leukemia Group: 30 years of research and achievements. Eur J Cancer 2002;38 Suppl 4:S44–9.
94. Cooley L, Dendle C, Wolf J, et al. Consensus guidelines for diagnosis, prophylaxis and management of Pneumocystis jirovecii pneumonia in patients with haematological and solid malignancies, 2014. Intern Med J 2014;44(12b):1350–63.
95. Hunger SP, Lu X, Devidas M, et al. Improved survival for children and adolescents with acute lymphoblastic leukemia between 1990 and 2005: a report from the children's oncology group. J Clin Oncol 2012;30(14):1663–9.

96. Barry E, DeAngelo DJ, Neuberg D, et al. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute Acute Lymphoblastic Leukemia Consortium Protocols. J Clin Oncol 2007;25(7):813–9.
97. Ibrahim A, Ali A, Mohammed MM. Outcome of Adolescents with Acute Lymphoblastic Leukemia Treated by Pediatrics versus Adults Protocols. Adv Hematol 2014;2014:697675.
98. Haupt R, Valsecchi MG, Silvestri D, et al. Early and late deaths after elective end of therapies for childhood cancer in Italy. Int J Cancer 2000;86(3):393–8.
99. Ethier M-C, Blanco E, Lehrnbecher T, Sung L. Lack of clarity in the definition of treatment-related mortality: pediatric acute leukemia and adult acute promyelocytic leukemia as examples. Blood 2011;118(19):5080–3.
100. Alexander S, Pole JD, Gibson P, et al. Classification of treatment-related mortality in children with cancer: a systematic assessment. Lancet Oncol 2015;16(16):e604–10.

VIII. APPENDIX

Appendix 1. Description of treatment related deaths reported by other groups

DEATHS DUE TO TOXICITY IN ACUTE LYMPHOBLASTIC LEUKEMIA PEDIATRIC TRIALS							
GROUP (Reference)	**STUDY TYPE**	**POPULATION**	**TRIAL(S)**	**TIME PERIOD**	**NUMBER OF PATIENTS [a] (% Deaths in Induction-First Remission)**	**TREATMENT RELATED DEATH (%)**	**CAUSES DEATH (Total)**
MRC (Atra A. et al)	Retrospective Single institution (Hospital Sick Children, London, England)	**Age:** Adults-Children (II and IV) 1-14 years (III) 1-14 years (PLOD) 1-15 years (X)	MRC UKALL II-IV PLOD MRC UKALL X	**1972**-1978 1979-1982 1983-**1989**	298 (3.7-6) 214 (1.9-4.7) 330 (0.6-5.2) **Total: 842**	DBT/Induction: 17 (2)	Infection[b]:6/0/0/1 (7) Bleeding/Thrombosis: 3/0 Tumor burden[c]:2 ALL progression: 5
						Early death (ED): 17 (2)	Early death (ED): 17
						During or After treatment: 35/7 (4.1/0.8) HSCT: 3 (0.4)	Infection:9/17/1/2/7 (36) TRM: 3 SMN: 4 Others[d]: 2
						Death in first CR (DCR): 45 (5.3)	Death in first CR (DCR): 45
						Overall: 62 (7.4)	
MRC (Hargrave D. et al)	Retrospective Multi institutional cooperative (United Kingdom)	**Age:** < 14 years (VIII) 1-15 years (X-XI)	MRC UKALL VIII MRC UKALL X MRC UKALL XI	**1980**-1984 1985-1990 1991-**1997**	829 (2.8-6) 1612 (2.4-2.6) 2090 (1.2-1.3) **Total: 4531**	DBT/Induction: 87 (1.9)	Infection: 42/0/14/0/0 (56) Bleeding/Thrombosis: 15/1 Tumor burden: 8 Others[e]:7
						Early death (ED): 87 (1.9)	Early death (ED): 87
						During or After treatment: 104 (2.3) HSCT: 18 (0.4)	Infection: 24/34/11/13/0 (82) TRM: 18 Others: 22
						Death in first CR (DCR): 122 (2.7)	Death in first CR (DCR): 122
						Overall: 209 (4.6)	

MRC (Mitchel C. et al)	Retrospective Multi institutional cooperative (United Kingdom)	**Age:** < 14 years (VIII) 1-15 years (X-XI-ALL97/99)	MRC UKALL VIII MRC UKALL X MRC UKALL XI	**1980**-1984 1985-1990 1991-1997 1997-2002	825 (*-7) 1612 (*-4) 2090 (*-2) 1935 (*-4) **Total: 6462**	DBT/Induction: - Early death (ED): During or After treatment: 232 HSCT: - Death in first CR (DCR): 232 (3.6) **Overall: 232 (3.6)**	During or After treatment: 191 Second neoplasms: 41 Death in first CR (DCR): 232
JCCLSG (Tsurusawa M. et al)	Retrospective Multi institutional cooperative (Japan)	**Age:** ≤ 18 years	ALL 811 ALL 841 ALL 847 ALL 911	**1981**-1984 1984-1987 1987-1990 1991-**1993**	207 (*-1) 220 (*-0.5) 371 (*-0.8) 223 (*-4) **Total : 1021**	DBT/Induction:- Early death (ED): - During or After treatment: 15 (1.5) HSCT: - Death in first CR (DCR): 15 (1.5) **Overall: 15 (1.5)**	Early death (ED): - Infection: 12 Others: 3 Death in first CR (DCR): 15
TCCSG (Tsuchida M. et al)	Retrospective Multi institutional cooperative (Japan)	**Age:** 1-18 years	L81-10 L84-11 L89-12 L912-13	**1981**-1984 1984-1989 1989-1992 1992-**1995**	189 (*-*) 484 (*-*) 418 (*-*) 347 (*-*) **Total: 1438**	DBT/Induction: 24 (1.7) Early death (ED): 24 (1.7) During or After treatment: 41 (2.9) HSCT: - () Death in first CR (DCR): (2.9) **Overall: 65 (4.5)**	-

DFCI (Silverman LB. et al)	Retrospective Multi institutional cooperative (DFCI)	**Age:** ≤ 18 years	DFCI 81-01 DFCI 85-01 DFCI 87-01 DFCI 91-01 DFCI 95-01	**1981**-1985 1985-1987 1987-1991 1991-1995 1996-**2000**	289 (*-*) 220 (0.5-3.6) 369 (2.2-1.9) 377 (0.5-3.2) 491 (0.8-0.6) **Total: 1746**	DBT: - Induction: 11 (0.6) Early death (ED): 11 (0.6) During/After treatment: 40 (2.3) HSCT: - Death in first CR (DCR)[f]: 40 (2.3) **Overall: 51 (2.9)**	-
BFM (Schrappe M. et al)	Retrospective Multi institutional cooperative (BFM)	**Age:** ≤ 18 years	ALL-BFM 81 ALL-BFM 83 ALL-BFM 86 ALL-BFM 90	**1981**-1983 1983-1986 1986-1990 1990-**1995**	611 (*-*) 653 (*-*) 998 (*-*) 2178 (*-*) **Total: 4440**	DBT: - Induction: 47 (1.1) Early death (ED): 47 (1.1) During/After treatment: 74 (1.7) HSCT: - Death in first CR (DCR): 74 (1.7) **Overall: 121 (2.8)**	-
Austrian BFM Study Group (Prucker C. et al)	Retrospective Multi institutional cooperative (BFM-Austria)	**Age:** ≤ 18 years	ALL-BFM (A) 81 ALL-BFM (A) 84 ALL-BFM (A) 86 ALL-BFM (A) 90 ALL-BFM (A) 95	**1981**-1983 1983-1986 1986-1990 1990-1995 1995-**1999**	141 (2.2-*) 127 (2.2-*) 142 (0.2-*) 256 (0.2-*) 230 (0.2-*) **Total: 896**	DBT: - Induction: 7 Early death (ED): 7 (0.8) During/After treatment: 24 HSCT: - Death in first CR (DCR): 24 (2.7) **Overall: 31 (3.5)**	Infection: 2/0/1 (3) Bleeding/Thrombosis: - Tumor burden: - Others: 4 Early death (ED): 7 Infection: 2/2/2/3/8 Toxicity: 1 Bleeding/Thrombosis: 1/1 Others: 4 Death in first CR (DCR): 24

COALL (Harms DO. et al and Escherich G. et al)	Retrospective Multi institutional cooperative (COALL)	**Age:** ≤ 18 years (In trials **89-92**, **infants** were treated according to a different regimen and included in the analysis)	COALL-82 COALL-85 COALL-89 COALL-92 COALL-97	**1982-**1985 1985-1989 1989-1992 1992-1997 1997-**2003**	129 (*-3.9) 289 (*-2.1) 205 (*-3.9) 519 (*-2.3) 667 (*-2.7) **Total: 1818**	DBT: - Induction: 5 (0.3) Early death (ED): 5 (0.3) During/After treatment: 49 (2.7) HSCT: - Death in first CR (DCR): 49 (2.7) **Overall: 54 (3)**	-
AIEOP (Conter V. et al)	Retrospective Multi institutional cooperative (Italy)	**Age:** 1-15 years (**Infants** were only included in Studies **87-88**)	Study 82 Study 87 Study 88 Study 91 Study 95	**1982-**1987 1987-1991 1988-1992 1991-1995 1995-**2000**	902 (2.2-3.3) 632 (0.6-1.6) 396 (1.3-1.8) 1192 (1.3-1.9) 1743 (0.7-1.6) **Total: 4865**	DBT/Induction: 57 (1.2) Early death (ED): 57 (1.2) During or After treatment: 97 (2) HSCT: - () Death in first CR (DCR): 97(2) **Overall: 154 (3.2)**	-
CCG (Gaynon PS. et al)	Retrospective Multi institutional cooperative (COG)	**Age:** <21	CCG 100 series CCG 1800 series CCG 1900 series	**1983**-1988 1989-1995 1996-**2002**	3713 (0.8-1.1) 5121 (0.7-1.8) 4464 (0.9-2.2) **Total : 13298**	DBT: - Induction: 107 (0.8) Early death (ED): 107 (0.8) During/After treatment: 230 (1.7) HSCT: - Death in first CR (DCR): 230 (1.7) **Overall: 337 (2.5)**	-

DCOG (Slats AM. et al)	Retrospective Multi institutional cooperative (Net herla nds)	**Age :** <16	DCOG ALL-6 DCOG ALL-7 DCOG ALL-8	**1984**-1988 1988-1991 1991-1996	190 (1.6-1.6) 218 (0.9-2.8) 467 (0.9-1.1) **Total: 875**	DBT: 3 (0.3) Induction: 6 (0.7)	Infection: 1 Bleeding/Thrombosis: 5/0 Tumor burden: 1 Others: 2
						Early death (ED): 9 (1)	Early death (ED): 9
						During/After treatment: 9 (1) HSCT: 5 (0.6)	Infection: 2/0/1/1/3(7) TRM: 5 Bleeding: 1 Others: 1
						Death in first CR (DCR): 14 (1.6)	Death in first CR (DCR): 14
						Overall: 23 (2.6)	
DCOG (Kamps WA. et al)	Retrospective Multi institutional cooperative (Netherlands)	**Age :** <16	DCOG ALL-9	**1997-2004**	**Total : 859 (1-2.2.6)**	DBT: 1 (0.1) Induction: 9 (1)	Infection: 5/0/1 (6) Tumor burden: 1 Others: 2
						Early death (ED): 10 (1.2)	Early death (ED): 10
						During/After treatment: 20 (2.3) HSCT: 3 (0.3)	Infection: 15 TRM: 3 Others: 5
						Death in first CR (DCR): 23 (2.6)	Death in first CR (DCR): 23
						Overall: 33 (3.8)	
St. Jude (Rubnitz J. et al)	Retrospective Single institution (St. Jude, Memphis, USA)	**Age:** ≤ 18 years	Total Therapy Studies XI-XII-XIIIA-XIIIB-XIV	1984-1999	**Total: 1011 (1.4-2.1)**	DBT: 0 Induction: 14 (1.4)	Infection: 1/2/8/0/0 (11) Bleeding/Thrombosis: 0 Others: 3
						Early death (ED): 14 (1.4)	Early death (ED): 14
						During/After treatment: 10/6 (1.6) HSCT: 6 (0.6)	Infection[h]:7/2/1/0/0 (9) TRM: 6 Others: 7
						Death in first CR (DCR): 22 (2.1)	Death in first CR (DCR): 22
						Overall: 36 (3.6)	

POG (Salzer WL. et al)	Retrospective Multi institutional cooperative (POG)	**Age:** ≤ 21	**ALinc14-15-16** (B-Precursor) **8704-9404** (T-Precursor) **8398-8493-9107** (Infant ALL)	1986-1999 1987-2001 1984-1993	6466 (0.8-1.7) 705 (1.7-2.4) 164 (5.5-7.3) **Total: 7335**	DBT: - Induction: 70 Early death (ED): 70 (1) During/After treatment: 142 (1.9) HSCT: - Death in first CR (DCR): 142 (1.9) **Overall: 212 (2.9)**	-
INS (Stark B. et al)	Retrospective Multi institutional cooperative (INS)	**Age :** ≤ 21	INS 84 INS 89 INS 98	1984-1989 1989-1997 1998-2003	134 (0-1.5) 337 (0-3.2) 315 (2.2-2.2) **Total : 786**	DBT: - Induction: 7 (0.9) Early death (ED): 7 (0.9) During/After treatment: 20 (2.5) HSCT: - Death in first CR (DCR)[i]: 20 (2.5) **Overall: 27 (3.4)**	- Early death (ED): 7 Infection: 18 Others: 2 Death in first CR (DCR): 20
CPH-BFM (Stary J, et al)	Retrospective Multi institutional cooperative (CPH)	**Age:** ≤ 18 years	ALL-BFM 90 ALL-BFM 95	1990-1996 1996-2002	350 (4-5.4) 380 (3.9-3.7) **Total: 730**	DBT: - Induction: 29 (4) Early death (ED): 29 (4) During/After treatment: 33 HSCT: - Death in first CR (DCR)[j]: 33 (4.5) **Overall: 62 (8.5)**	- Early death (ED): 7 Infection: 25 Others: 8 Death in first CR (DCR): 33

NOPHO (Christensen MS. et al and Lund B M. et al)	Retrospective Multi institutional cooperative (NOPHO)	**Age:** 1-15 years	NOPHO-ALL 92 NOPHO-ALL 2000	1992-2001 2002-2007	1645 (1-2) 1090 (1.2-1.8) **Total: 2735**	DBT: 5 (0.2) Induction: 34 (1.2)	Infection: 26 Bleeding/Thrombosis: 6 Tumor burden: 7
						Early death (ED): 39 (1.4)	Early death (ED): 39
						During/After treatment: 39 (1.4) HSCT: 10 (0.4)	Infection: 30 TRM: 10 Toxicity: 6 Others: 3
						Death in first CR (DCR): 49 (1.8)	Death in first CR (DCR): 49
						Overall: 88 (3.2)	
TPOG (Liang D-C. et al)	Retrospective Multi institutional cooperative (Taiwan)	**Age:** ≤ 15 years	TPOG-93	**1993-1997**	**Total : 201 (3-3.5)**	Induction: 6 (3)	Infection: 5 Bleeding/Thrombosis: 1
						Early death (ED): 6 (3)	Early death (ED): 6
						During or After treatment: 7 (3.5) HSCT: -	Infection: - Others: -
						Death in first CR (DCR): 7 (3.5)	Death in first CR (DCR): 7
						Overall: 13 (6.5)	
TPOG (Liang D-C. et al)	Retrospective Multi institutional cooperative (Taiwan)	**Age:** ≤ 18 years	TPOG-97 TPOG-2002	**1997**-2001 2002-**2007**	602 (*-6) 788 (*-0.6) **Total : 1390**	DBT/Induction:-	
						Early death (ED): -	Early death (ED): -
						During or After treatment: 41 (2.9) HSCT: -	Infection: 35 Others: 6
						Death in first CR (DCR): 41 (2.9)	Death in first CR (DCR): 41
						Overall: 41 (2.9)	

ALL: Acute lymphoblastic leukemia; **CR:** Complete remission; **MRC:** Medical Research Council; **JCCLSG:** Japanese Childhood Cancer and Leukemia Study Group; **TCCSG:** Tokyo Children's Cancer Study Group; **DFCI:** Dana-Farber Cancer Institute; **BFM:** Berlin-Frankfurt-Munich; **COALL:** German Co-operative study group for treatment of acute lymphoblastic leukemia in childhood; **AIEOP:** Associazione Italiana di

Ematologia ed Oncologia Pediatrica; **CCG:** Children´s cancer group; **DCOG:** Dutch Childhood Oncology Group; **POG:** Pediatric Oncology Group; **INS:** Israel National Studies in childhood ALL; **CPH:** Group for Pediatric Hematology in Czech Republic; **NOPHO:** Nordic society of paediatric haematology and oncology; **TPOG:** Taiwan Pediatric Oncology Group; **CR:** Complete remission; **DBT:** Death before treatment; **ED:** Early death; **HSCT:** Hematopoietic stem cell transplantation; **DCR:** Death in first complete remission; **TRM:** Transplant in first CR related mortality; **SMN:** Secondary malignant neoplasm

[a] Number of patients included only eligible and evaluable patients who were finally included in the analysis in each trial by any single group. In brackets the percentage of deaths during induction and in first complete remission for each trial are depicted when this information could be extracted from the paper. This missing information is represented by *.*.

[b] Infections have been divided in 5 groups and separated by slashes: Bacterial/Viral/Fungal/*Pneumocystis carinii*/Others or unknown.

[c] Tumor burden related deaths included tumor lysis syndrome (TLS) and leukostasis with compromised organ function due to infiltrating blast cells (e.g. mediastinal mass).

[d] Others in the group of DCR included adult respiratory distress syndrome (ADRS), aspiration, cardiac arrhythmias, sudden death, traffic accidents, suicide, iatrogenic causes and unknown causes.

[e] Others in the group of ED included non infectious neurological insults (stroke, cerebral edema, brain hernia, encephalopathy), iatrogenic and unknown causes.

[f] Deaths in CR were primarily due to infectious complications as explained in the paper (No numeric values provided). No information about death during induction to remission could be figured out from this report.

[g] Deaths during induction were mostly due to infections.

[h] One patient in this study had disseminated Varicella and *Candida* infections.

[i] Secondary neoplasms were reported in this paper. Nevertheless, the outcome of this group of patients could not be figure out and therefore not plotted in the chart.

[j] Secondary neoplasms were reported in this paper. Nevertheless, the outcome of this group of patients could not be figure out and therefore not plotted in the chart.

Appendix 2. Detailed description of the patients who died of toxicity on each EORTC protocol

Table A. Types of infective deaths in EORTC-CLG trials											
	Early deaths (ED) DBT/Prephase/Induction			Deaths in remission				Hematopoietic stem cell transplantation			Total (%)
Type organism / Trial	**58831/2**	**58881**	**58881**	**58741**	**58831/2**	**58881**	**58951**	**58831/2**	**58881**	**58951**	
Bacterial	**9**	**6**	**13**	**2**	**0**	**8**	**7**	**0**	**0**	**1**	**46 (44.7)**
Pseudomonas spp.	3	3	2	0	0	0	1	0	0	0	9
Escherichia Coli	1	0	3	1	0	1	2	0	0	0	8
Klebsiella	1	0	0	1	0	0	0	0	0	0	2
Streptococcus	4	3	2	0	0	0	0	0	0	0	9
Staphylococus	0	0	1	0	0	1	0	0	0	0	2
Mycoplasma	0	0	0	0	0	1	0	0	0	0	1
Enterobacter	0	0	1	0	0	1	0	0	0	0	2
Proteus	0	0	1	0	0	0	1	0	0	0	2
Enterococus	0	0	0	0	0	0	1	0	0	0	1
Lysteria	0	0	0	0	0	1	0	0	0	0	1
Unknow bacteria	0	0	0	0	0	3	1	0	0	1	5
Viral	**0**	**0**	**0**	**4**	**3**	**4**	**2**	**0**	**3**	**3**	**19 (18.4)**
Varicella	0	0	0	4	0	0	1	0	0	1	6
Measles	0	0	0	0	2	2	0	0	0	0	4
HSV	0	0	0	0	0	0	0	0	1	0	1
EBV	0	0	0	0	0	1	0	0	1	1	3
Echo-Virus 6	0	0	0	0	1	0	0	0	0	0	1
HBV	0	0	0	0	0	0	1	0	0	0	1
Adenovirus	0	0	0	0	0	0	0	0	0	1	1
Papilomavirus	0	0	0	0	0	0	0	0	1	0	1
Influenza	0	0	0	0	0	1	0	0	0	0	1
Fungal	**3**	**1**	**3**	**0**	**1**	**7**	**4**	**0**	**5**	**0**	**24 (23.3)**
Aspergillus	3	1	3	0	1	4	3	0	4	0	19
Candida	0	0	0	0	0	2	1	0	1	0	4
Unknown fungal	0	0	0	0	0	1	0	0	0	0	1
Pneumocystis carinii	**0**	**0**	**0**	**4**	**0**	**0**	**1**	**0**	**0**	**0**	**5 (4.9)**
Toxoplasma	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**2 (1.9)**
Unknown	**0**	**3**	**0**	**1**	**0**	**0**	**2**	**0**	**1**	**0**	**7 (6.8)**
Total	**12**	**10**	**16**	**11**	**4**	**19**	**16**	**0**	**11**	**4**	**103 (100)**

Table B. Types of non-infective deaths in EORTC-CLG trials								
	Early deaths (ED) DBT/Prephase/Induction			Deaths in remission				Total (%)
Death group / Trial	**58831/2**	**58881**	**58951**	**58741**	**58831/2**	**58881**	**58951**	
Tumor burden	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**3 (3.1)**
TLS	0	0	0	0	0	0	0	0
Leukostasis	2	1	0	0	0	0	0	3
Bleeding / Thrombosis	**5**	**5**	**1**	**0**	**0**	**2**	**2**	**15 (15.6)**
Intracranial hemorrhage	3	3	0	0	0	0	0	6
DIC	2	0	0	0	0	0	0	2
Gastrointestinal hemorrhages	0	2	1	0	0	0	0	3
Pulmonary hemorrhage	0	0	0	0	0	0	1	1
Thrombosis	0	0	0	0	0	2	1	3
Organ toxicity	**0**	**0**	**0**	**3**	**0**	**6**	**0**	**9 (9.4)**
Pancreatitis	0	0	0	0	0	1	0	1
Cardiotoxicity	0	0	0	0	0	2	0	2
Hepatic toxicity	0	0	0	3	0	1	0	4
Others	0	0	0	0	0	2	0	2
TRM	**0**	**0**	**0**	**0**	**2**	**22**	**7**	**31 (32.3)**
GVHD	0	0	0	0	0	7	2	9
Infection	0	0	0	0	0	11	4	15
Conditioning regimen toxicity	0	0	0	0	2	0	0	2
Others	0	0	0	0	0	4	1	4
Secondary neoplasms	**0**	**0**	**0**	**1**	**0**	**11**	**3**	**15 (15.6)**
AML	0	0	0	0	0	7	2	9
Sarcomas	0	0	0	0	0	1	0	1
Brain tumors	0	0	0	1	0	0	1	2
Others	0	0	0	0	0	3	0	3
ALL progression	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**2 (2.1)**
Others	**1**	**3**	**0**	**0**	**3**	**6**	**8**	**21 (21.9)**
Necrotising enterocolitis	1	0	0	0	0	0	0	**1**
VOD	0	1	0	0	0	0	0	**1**
ARDS	0	2	0	0	0	0	2	**4**
Iatrogenia	0	0	0	0	1	1	0	**2**
Histiocytic disorders	0	0	0	0	2	2	2	**6**
Others	0	0	0	0	0	3	4	**7**
Total	**9**	**10**	**1**	**4**	**5**	**47**	**20**	**96 (100)**

ALL: Acute lymphoblastic leukemia; **AML:** Acute myeloid leukemia; **ARDS:** Acute respiratory distress syndrome; **DBT:** Death before treatment; **DIC:** Disseminated intravascular coagulation; **EBV:** Epstein Barr Virus; **GVHD:** Graft versus host Disease**; HSCT:** Hematopoietic stem cell transplantation; **HBV:** Hepatitis B Virus; **HSV:** Herpes simplex virus; **TLS:** Tumor lysis syndrome; **TRM:** Transplant related mortality; **VOD:** Veno-occlusive disease.

Appendix 3. Cumulative doses of chemotherapy of the EORTC-CLG ALL protocols

Cumulative doses of chemotherapy – All protocols								
Drug/Trial	**58741**	**58831**	**58832**	**58881**		**58951**		
	All patients	**Standard Risk**	**Medium/High Risk**	**Standard Risk**	**Very High Risk**	**Very Low Risk**	**Average Risk (AR1 – AR2)**	**Very High Risk**
BCNU (mg/m^2)	50	-	-	-	-	-	-	-
Daunorubicin (mg/m^2)	120	120	120	120	220	60	120-90	240
Doxorubicin (mg/m^2)	-	60	120	120	-	60	120	-
Mitoxantrone (mg/m^2)	-	-	-	-	16	-	-	-
Cyclophosphamide (mg/m^2)	140	0 Vs 2000 (According to randomization)	3000	3000	2000	-	3000-4000	3000
Ifosfamide (mg/m^2)	-	-	-	-	800	-	-	-
L-Asparaginase (IU/m^2)	600000	145000	145000	120000	345000	120000 Vs 160000 (According to randomization)	120000 Vs 160000 (According to randomization)	250000
Vincristine (mg/m^2)	20	9	12	12	8	12	12	14
Cytarabine (mg/m^2)	-	1800	2400	1800 Vs 9800 (According to randomization)	22000	1800	1800	18000
Etoposide (mg/m^2)	-	-	-	-	900	-	-	900
IV Methotrexate (g/m^2)	-	2	10	20	50	20	20-55	40
IT Methotrexate (Injections/Age dependant)	-	6	6	10	10	6	1	1
Triple IT (Injections/Age dependant)	-	-	-	-	6	4	16	15